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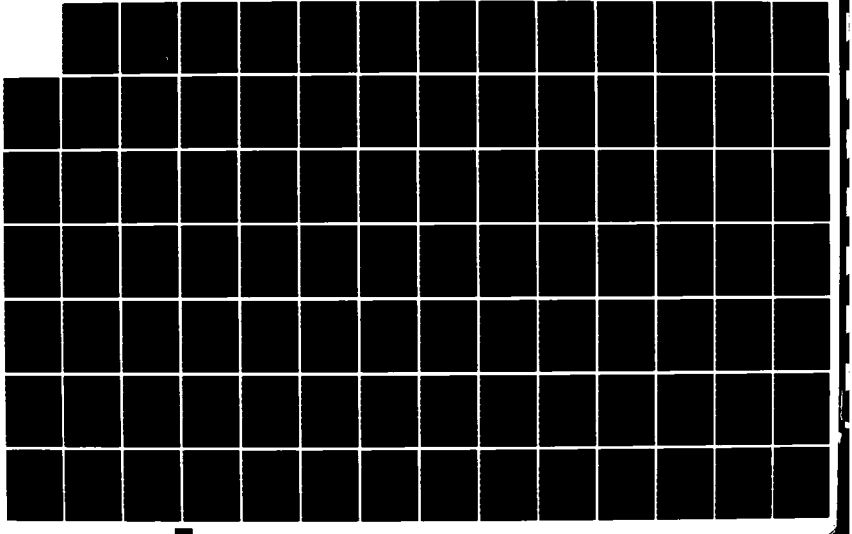

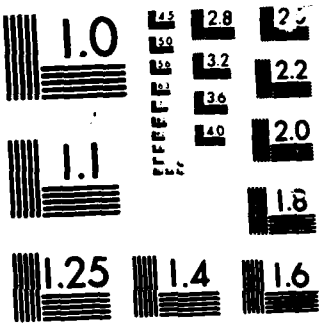

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TOXICOLOGIC EVALUATION OF TRICHLOROACETIC ACID: EFFECTS ON RAT LIVER PEROXISOMES AND ENZYME ALTERED FOCI

Abstract

by Michael Jesse Parnell, DVM, PhD
Washington State University
May 1986

Chair: Loren D. Koller

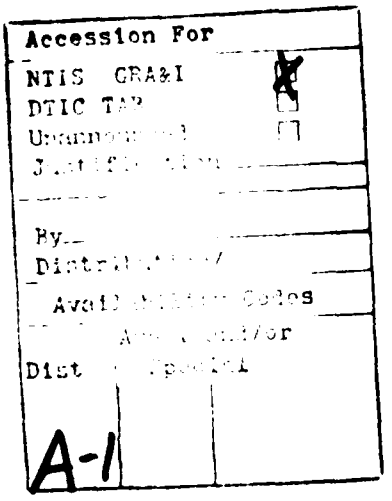
Trichloroacetic acid (TCA) is one of the major non-volatile halogenated by-products formed during the process of water chlorination. Recent studies have indicated that TCA may be an effective stimulant of hepatic peroxisomal activity in the rat and mouse. It has been further suggested that TCA, a metabolite of trichloroethylene, may be involved in the hepatic carcinogenic effect of trichloroethylene. This research was designed to determine the relative potential of low-levels of TCA to produce toxic effects, including carcinogenicity, in an animal model.

The initiating and promoting effects of TCA were investigated using a rat hepatic enzyme-altered foci (EAF) bioassay. No evidence was found to support any significant genotoxic or initiating activity by TCA. However, a significant induction of EAF in the promotion protocol was seen following TCA exposure.

cont'd

The ability of TCA to stimulate peroxisomal-dependent palmitoyl-CoA oxidation was also investigated. Only the high dose (5000 ppm) in the drinking water resulted in a significant, though minor, stimulation of peroxisomal enzyme activity. A single high dose (1500 mg/kg) of TCA did stimulate a large increase in hepatic ornithine decarboxylase activity when measured at 5 and 18 hours post exposure. No significant changes in hepatic mixed function oxidase activity was seen following TCA administration. No consistent or significant pathologic lesions could be attributed to TCA exposure at any phase of these studies.

The findings support the hypothesis that TCA may possess weak promoting activity in the rat liver.



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TOXICOLOGIC EVALUATION OF TRICHLOROACETIC ACID: EFFECTS ON RAT LIVER PEROXISOMES AND ENZYME-ALTERED FOCI

By

MICHAEL JESSE PARNELL

A dissertation submitted in partial fulfillment of
the requirements for the degree of

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of MICHAEL JESSE PARNELL find it satisfactory and recommend that it be accepted.

Chair

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Dedication

To the four most important people in my life:
Chris, Jennifer, Erin, and Jamie

I. INTRODUCTION

Water, the universal biological solvent, is essential for life as we know it. The importance of a clean, reliable source of drinking water has always been apparent to man as an individual and to societies as a whole. While modern man often takes the availability of "good" water for granted, outbreaks of waterborne disease occur frequently enough, even in the U.S., that considerable effort is required to maintain adequate potable water supplies.

Since its first use in the United States, in 1908 in Jersey City, the disinfection of drinking water with chlorine has become commonplace. The effectiveness of chlorination has been repeatedly demonstrated by empirical measurements of pre- and post-treatment levels of waterborne pathogens. The rapid decline of deaths in the early part of this century due to typical waterborne diseases, such as typhoid fever, also clearly showed the importance of water disinfection. Most public health experts feel that all municipal water supplies should be chemically disinfected. Currently in the U.S., water disinfection is practically synonymous with chlorination. Besides treatment of drinking water, chlorine is the most commonly used chemical to control biofouling at power plants, to disinfect wastewater from sewage treatment plants, and to bleach paper pulp. Thus, the sources for

environmental contamination of both fresh and salt water with chlorinated compounds are already numerous and steadily growing.

Only within the last decade have possible health hazards arising from water chlorination been recognized. The observation that chloroform, an animal carcinogen, and other trihalomethanes (THM) are produced as by-products of chlorination resulted in the EPA setting drinking water standards for THM in 1979. Further investigations have shown that numerous other chlorination by-products, more difficult to detect and analyze than the THM, can be found in treated water. These by-products result primarily from the interaction of the added chlorine with naturally occurring organic compounds present in the water. Chlorine interaction with industrial and domestic organic water contaminants, also produces halogenated by-products.

Many of the halogenated by-products of chlorination are mutagenic in bacterial assays. Several epidemilogic studies have indicated a possible association between the consumption of chlorinated drinking water and various forms of cancer in humans. These and other possible health hazards have caused the current reevaluation of the risk-benefit for disinfection of drinking water with chlorine.

Within the last five years, trichloroacetic acid, dichloroacetic acid, and chloral have been identified as major by-products of water chlorination. Trichloroacetic acid is consistantly found in chlorinated drinking water at concentrations which often exceed the level of chloroform. Very little data is available concerning possible biological effects following chronic low-level exposure to trichloroacetic acid and other chlorination by-products.

This research was designed to determine the relative potential of low-levels of trichloroacetic acid to produce toxic effects, including carcinogenicity, in an animal model. Data concerned with the biological effects of trichloroacetic acid and several metabolically related compounds suggest the liver as a target organ. Most recently, the reported hepatic peroxisomal effects of trichloroacetic acid indicate it may either initiate or promote neoplasia in rodents.

The hypothesis tested was that trichloroacetic acid is capable of inducing and/or modulating hepatic carcinogenesis in the rat. The experimental approach was to emphasize the possible hepatic carcinogenic effects while assessing the potential for general toxicity. The questions addressed were: (1) Does trichloroacetic acid initiate or promote liver carcinogenesis, as measured by short term initiation-promotion assays?; (2) What are the general toxic effects of subchronic or chronic exposure to

trichloroacetic acid measured by serochemistry, hepatic mixed-function oxidase induction, organ/body weight, and histopathology?; and, (3) Is the liver a specific target organ for trichloroacetic acid induced toxicity?

II. LITERATURE REVIEW

A. Trichloroacetic Acid

1. Background

Trichloroacetic acid (TCA) is one of a large group of chemicals formed as by-products during chlorination of drinking water which contains organic material (1-5). These halogenated reaction products can be separated into volatile compounds, purgable from water at ambient temperatures, and nonvolatile halogenated products. In 1974 Rook, demonstrated that chlorination of water containing natural humic substances and inorganic bromide led to the formation of the volatile trihalogenated methanes (THM), chloroform, bromoform, bromodichloromethane, and dibromochloromethane (6). Chloroform is the most common THM formed during chlorination (2,3,6). The National Cancer Institute, in 1976, published results showing that at high doses chloroform could cause cancer in rodents (7). This information caused the Environmental Protection Agency (EPA) to limit the amount of chloroform in finished

drinking water to 100 ug/L (8). Chloroform and other volatile chlorinated organics were considered to be the major halogenated organic by-products formed during the chlorination of surface waters (9).

More recently, studies have shown that there are many nonvolatile organic reaction products formed during the chlorination process (1-5,10,11). Generally these chemicals have been more difficult than the trihalomethanes to identify in drinking water, partially because they are more polar and because the bulk of the organic matter in drinking water is relatively nonvolatile (2). With the development of better analytical methods, it has been shown, under practical treatment-plant conditions, that the formation of nonvolatile organic-bound chlorine exceeds the volatile fraction by a factor of 2 to 5 (11,12). Chromatographic analysis has shown TCA and dichloroacetic acid (DCA) to be the major nonvolatile chlorinated organic by-products (1-4).

2. Physical and Chemical Properties

Trichloroacetic acid is a chlorinated, aliphatic carboxylic acid with a molecular formula of $C_2HCL_3O_2$ and a molecular weight of 163.40 daltons. TCA is soluble in 0.1 part water, highly soluble in alcohol and ether, forms a strong acidic aqueous solution (Ka = 0.232), and is deliquescent and white in its pure crystalline form. It is commercially prepared either by the oxidation of chloral

hydrate with nitric acid or through the chlorination of acetic acid. The stability of TCA in aqueous solution is good at room temperatures and below. Hydrolytic decomposition products of TCA are chloroform, hydrochloric acid, carbon dioxide and carbon monoxide (13,14).

3. Use and Environmental Sources

Approximately, 2.3 X 10^6 grams of TCA are produced commercially in the United States annually (15). The commercial uses of TCA are many and varied. In agriculture, the sodium salt of TCA is used as a pre-emergence herbicide to control weeds in sugarbeets, sugarcane, alfalfa, and rape (16). TCA does not persist in soil, plants, or water; therefore, its agricultural use has a low potential for environmental pollution (17,18). In medicine, TCA is primarily used as a caustic agent for treatment of the dermal lesions of acne, warts, lichenified eczema, tinea versicolor, chloasma, and molluscum contagiosum (19,20). It is also used, as an astringent drug to treat hyperhidrosis (19). Experimentally, TCA has been used to treat epithelial cysts of the anterior chamber of the eye and as a cautery agent for treating various corneal lesions. As a common laboratory reagent, TCA is widely used to precipitate protein and as a tissue fixative or decalcifier in microscopy (13). None of these direct uses of TCA are,

however, considered major sources of environmental contamination and human exposure.

The presence of TCA in treated drinking water has been confirmed by various researchers (1-5). The major non-volatile halogenated organic by-products of water chlorination appear to be TCA, along with (DCA) and chloral hydrate. (1-5). The concentrations of TCA in treated water that contains humic substances often exceeds the levels of chloroform (12,21). Available data indicate that the concentrations of TCA in drinking water range from ten to several hundred ppb (1,2). The environmental levels of TCA and the other nonvolatile chlorinated products vary with local conditions and are directly related to the concentration of humic materials (fulvic acid, humic acid and hymatomelanic acid) present in the water (1,5). While TCA and DCA are structurally similar, chlorination studies of fulvic and humic acids in the laboratory indicate that TCA formation does not proceed through a DCA intermediate, but that both form independently (1). The relative concentrations of each are dependent upon the reaction conditions (1,22). Enteric production of TCA and DCA following oral administration of sodium hypochlorite, a common source of chlorine for water treatment, has also recently been demonstrated (23).

Due to widespread use and indiscriminate disposal practices, many halogenated organic industrial chemicals

are now widely distributed in our nation's water supply (24-27). Trichloroethylene (TCE) is an organic solvent with wide industrial application and is a common contaminate of surface and ground water (24-28). Trichloroethanol along with TCA, are the major animal and human metabolites of TCE (29-32). Initially, TCE is oxidized to chloral hydrate, which is either reduced to trichloroethanol or oxidized to TCA. Biotransformation of chloral hydrate results in the formation of the same metabolites. Trichloroethane and tetrachloroethane are industrial chemicals which are also found as water pollutants and can be metabolized to TCA (24,27,30).

The nearly universal practice of chlorinating drinking water ensures widespread low level, chronic environmental exposure to TCA. Enteric formation following chlorine ingestion and possible water contamination with metabolically related halogenated organics also contribute to drinking water as the primary environmental source of TCA.

4. Pharmacokinetics

Only recently has the pharmacokinetics of TCA been examined (31,32). When ^{14}C-labeled TCA was given as an aqueous oral gavage at a dose of 75 mg/kg to rats, 7% of the dose was excreted as $^{14}CO_2$ in the expired air within the first 24 hours. Over the same period, 58% of the dose was excreted in urine and 1% in feces. Subsequently, rats

and mice, dosed intravenously with the radiolabeled TCA at 10 mg/kg, excreted 12% and 15% of the dose as $^{14}CO_2$, respectively, in the first 24 hours. Both rats and mice excreted approximately 35% of the iv dose in the urine.

These same investigators exposed rats and mice to trichloro[1,2-^{14}C]ethylene (^{14}C-TCE) orally in corn oil (31). In rats fitted with biliary cannula, 10% of the dose of ^{14}C-TCE was excreted in bile in the first 24 hours. Free TCA accounted for up to 14% of this radioactivity while the main metabolite was trichloroethanol glucuronide (78%). A low fecal excretion in noncannulated rats, together with the decrease in urinary excretion when a biliary cannula was fitted, suggested extensive enterohepatic circulation of the material excreted in bile. Similar data was obtained from mice (31).

It is unlikely that TCA, on a molecular weight basis, is excreted in the free form (33), which suggests that a labile conjugate is formed in the liver. Such a conjugate would account for the biliary excretion of TCA and the rapid reabsorption from the intestine that appears to occur (32). Also enterohepatic circulation could be a contributory factor to the long half-life of TCA in many species (34,35). Because it persists in human plasma for several days following TCE exposure, plasma TCA has been used as a biological marker of human exposure to TCE (36).

5. Toxicity

Toxicologically, TCA is classified as a corrosive organic acid. Most toxicological data on TCA deal with acute and chronic caustic effects on various organ systems and animal models. The toxic hazard for TCA as an irritant and for ingestion or inhalation is considered high (37). Acute human oral exposure is reported to cause severe oral and abdominal pain, vomiting, bloody diarrhea, rapid fall in blood pressure, and edema of the glottis resulting in asphyxia (38). Any systemic effects are presumably secondary to gastrointestinal damage and acidosis and not due to the trichloroacetate ion (39). The acute oral LD50 of TCA in rodents ranges from 3300 to 5600 mg/kg (16,40,41). Acute exposure of the eye or skin can result in hyperemia, burns, necrosis, and hemorrhage (38). Inhalation of aerosolized TCA results in coughing, choking, dizziness, and weakness; followed in 6 to 8 hours by chest tightness, air hunger, moist rales, lowered blood pressure, and death (38). Chronic inhalation has caused bronchitis, coughing, and pneumonia as well as erosion of the teeth and necrosis of the jaw (38).

Concentrations of TCA at 0.01, 0.1, and 1 ppm in filtered pond water caused significant increases in oxygen consumption and ammonia excretion rates in dragonfly nymphs (42).

6. Mutagenicity and Carcinogenicity

Some of the nonvolatile by-products, but not TCA, formed during water chlorination do exhibit mutagenic activity in the Ames assay (2,43-45). To date, no long-term bioassays have been performed which examine the carcinogenicity of TCA.

Lifelong, high-dose, oral exposure to TCE have produced hepatocellular carcinomas in mice, but not rats (46,47). More recently, oral TCE has been shown to stimulate hepatic peroxisomal proliferation in mice, but not rats (48,49). Peroxisomal proliferation has been linked with hepatic cancer in rodents (50-52). Oral exposure of rats and mice to TCA in corn oil at a dose of 50 mg/kg or greater, has been reported to lead to hepatic peroxisome proliferation (53). These data, and the recent kinetic studies in TCE-exposed rats and mice, indicate that the higher blood levels of TCA in mice, compared to rats, may explain the greater susceptibility of mice to TCE-elicited hepatocellular carcinomas (31,32,53).

B. Peroxisomes

1. Background

A single-membrane-limited organelle located in cytoplasm of a renal epithelial cell from the proximal convoluted tubule of a mouse was first termed a "microbody" by Rhodin, in 1954 (54). Later, these microbodies were

identified in the parenchymal cells of rat liver (55). In the 1960's several researchers showed that microbodies are present in liver and kidney cells of many animals (56-58). The presence of various oxidases and catalase within these microbodies was first shown by deDuve and co-workers (59-61). The term "peroxisome" was introduced by deDuve, in 1965, after it was shown that the oxidases in microbodies are capable of producing hydrogen peroxide as a reaction product (59). The development of a cytochemical procedure to localize catalase in 1968 made it possible to identify peroxisomes in many cells other than those of the kidney and liver (62). It is now well known that peroxisomes have a wide biological distribution and are found in both animal and plant cells (63-65).

2. Morphology

In hepatic parenchymal cells, peroxisomes appear as single-membrane-limited organelles which contain a finely granular electron-dense matrix (58). Hepatic peroxisomes measure approximately 0.3 to 1.0 um (56,58). A crystalloid core or nucleoid, which corresponds with the presence of urate oxidase, is seen in hepatic peroxisomes from many species (66). However, humans and several other species lack peroxisomal urate oxidase and the crystalloid core (56-58).

Peroxisomes are commonly identified within cells by use of the alkaline 3,3'-diaminobenzidine cytochemical

procedure developed in 1969, which demonstrates the presence of catalase (62). The peroxisomes present in tissues other than liver and kidney, which are identified using this cytochemical procedure, are called microperoxisomes. Microperoxisomes are smaller and lack a urate oxidase-containing nucleoid (63). Immunocytochemical procedures have also been developed for identification and ultrastructural localization of peroxisomes (67,68).

Differentiation of peroxisomes from other cytoplasmic organelles is usually easy. The peroxisome has a single limiting membrane, electron-dense homogeneous matrix, and no cristae, which allows differentiation from mitochondria. Peroxisomes are smaller than lysosomes, normally more uniform in appearance than lysosomes, are often closely associated with smooth endoplasmic reticulum, and don't contain vacuoles, lipid droplets, myelin figures, or ferritin (57). Cytochemical techniques can also be used to distinguish between lysosomes and peroxisomes (69,70).

3. Biochemical Characteristics

DeDuve was the first to biochemically characterize hepatic peroxisomes (61). Because catalase is consistently present within peroxisomes, it is used as a marker enzyme for peroxisomes from different sources (71). The peroxisomal oxidases appear to vary in number and specificity depending on the tissue source (72). Many different types of enzymes have been isolated from

peroxisomes, including hydrogen peroxide generating oxidases, acyl transferases, dehydrogenases, thiolase, fatty acyl-CoA synthetase, enoyl-CoA hydratase, and catalase (72).

Lazarow and deDuve first reported the existence of a peroxisomal pathway for B-oxidation of fatty acids in mammalian tissues in 1976 (73). Since that report, many research groups have studied the peroxisomal B-oxidation pathway for fatty acyl-CoA (72,74,75). The enzymes of the peroxisomal fatty acid B-oxidation system differ significantly from the enzymes of the mitochondrial system with respect to their molecular and catalytic properties (72,76).

4. Functions

Peroxisomes are involved in many different cellular functions. These include respiration, gluconeogenesis, lipid metabolism, thermogenesis, purine catabolism, and other miscellaneous metabolic functions (77). The peroxisomal respiratory pathway generates H_2O_2 and accounts for approximately 20% of the oxygen consumption of liver (60). The peroxisomal fatty acid oxidizing system is capable of oxidizing acyl-CoAs with 8 or more carbon chain lengths (78,79). Drugs and chemicals which are capable of inducing peroxisomal proliferation can increase the activity of the fatty acid oxidation system 7 to 15 times (80,81).

5. Peroxisomal Proliferation

During the last 10 to 15 years, several chemicals have been shown to be able to induce hepatic and renal peroxisomal proliferation (81,82). In the liver of rats and mice, these peroxisomal proliferators stimulate an increase in number and volume of peroxisomes and a corresponding hepatomegally (82-84). Changes in peroxisomal ultrastructural appearance are also induced, including variations in size and shape, and inclusions within the matrix (82,83).

The activity of many of the peroxisomal associated enzymes are significantly increased following exposure to peroxisomal proliferating agents (82). Catalase activity is increased, but not proportionally to the increases in peroxisomal number and volume (82,85). Activities of the enzymes of the peroxisomal fatty acid B-oxidation system are also increased several-fold over control values following exposure to peroxisomal proliferators (81,82).

The activity of peroxisomal B-oxidation can be measured either spectrophotometrically or by measuring the conversion of acid-insoluble [^{14}C]-palmitoyl-CoA to acid-soluble [^{14}C]-acetyl-CoA in homogenates or isolated organelles (86). With the more sensitive radioactivity

assay, a 6 to 15 fold increase in the peroxisomal B-oxidation activity has been seen in liver homogenates of rats, mice, and hamsters fed peroxisome proliferators (81,82).

The ability to induce peroxisomal proliferation has been associated with many different chemicals. The hypolipidemic drugs such as clofibrate and nafenopin are classic examples of peroxisomal proliferators (81,82). Besides hypolipidemic agents, many different phthalate ester plasticizers and compounds such as aspirin and tibric acid are classified as peroxisomal proliferators (81,82). Dietary manipulations, high fat diet and vitamin E deficiency also produce proliferation of hepatic peroxisomes (82).

It has been argued that the hypolipidemic drug-induced hepatic peroxisomal proliferation seen in rodents was peculiar to rodents (87,88). However, more recently, chemically induced peroxisomal proliferation has been shown to occur in many species including primates (89).

6. Carcinogenicity of Peroxisomal Proliferators

Reddy et al. has speculated that potent hepatic peroxisomal proliferators are a novel class of chemical carcinogens (90). Since this hypothesis was formulated,

many of the hypolipidemic drugs have been found to induce hepatocellular neoplasms in animals (87,91). Several industrial phthalate ester plasticizers are also known to induce liver cancer in rodents (92,93).

Selected carcinogenic hypolipidemic peroxisome proliferators have been evaluated for their mutagenic activity using both the the Salmonella/microsome (Ames) and lymphocyte [3H]thymidine assays (94). The hypolipidemics were uniformly negative as mutagens (94). Two plasticizers produced similar negative results in the Ames assay (95). No evidence is available to date that provides support for the mutagenic ability of carcinogenic peroxisome proliferators in in vitro systems.

The mechanism(s) through which potent peroxisomal proliferators induce hepatocellular carcinomas is not known. Studies have provided evidence that some of the peroxisomal proliferators possess tumor-promoting activity in diethylnitrosamine-initiated hepatic carcinogen assays in the rat (96-98). However, several of the peroxisome proliferators, when administered by themselves, can induce nearly 100% tumor incidence in rats and mice (99,100). This is in contrast with most known tumor promoters which are not carcinogenic or only weakly carcinogenic by

themselves (101-103). The available evidence indicates that peroxisome proliferators, like most complete carcinogens, possess both cancer initiating and promoting activity (101).

Currently, the most accepted hypothesis for carcinogenic mechanisms of peroxisome proliferators has been proposed by Reddy et al. They suggest that peroxisome proliferation in cells is associated with an increase in the rate of production, and possibly steady-state levels, of reactive oxygen species in the cells (90,94). It has been shown that H_2O_2 is formed in excess in the liver as a consequence of sustained peroxisome proliferation; this could result in the production of hydroxyl-radical by the Haber-Weiss reaction catalyzed by cellular iron (99,104-106). As mentioned previously, the increase in liver catalase activity is disproportionately small compared to the increase in peroxisome number, volume, and H_2O_2 generating fatty acid B-oxidation in peroxisomal proliferator- treated animals. This is consistent with the above hypothesis since peroxisomal catalase is one of the cellular defense mechanisms responsible for destroying most of the H_2O_2 formed in the organelle.

C. Chemical Carcinogenesis

1. Background

Cancer can be caused by many different agents. These include xenobiotics, radiation, viruses, physical agents,

and poorly defined "environmental factors." The single largest group of known carcinogenic agents are chemicals, both man-made and naturally occurring. Greater than 30 chemicals or mixtures are known to be involved in the genesis of cancer in human beings (107). In addition, an ever increasing number of environmentally important chemicals are being recognized as agents that can modulate human carcinogenesis (107). The well publicized link between smoking and lung cancer, the FDA ban on saccharin, the "agent orange" controversy, and many other recent events with a similar theme have led to a greater public awareness and interest in chemical carcinogenesis. This increasing interest has, in turn, induced much scientific effort toward identification of possible carcinogenic chemicals within our environment. It is possible with today's technology to identify agents that, under a specified set of conditions, will cause carcinogenic transformation of normal cells. However, the mechanisms involved in this transformation are still poorly understood. Until we have a good understanding of the molecular basis of the carcinogenic process, rational and highly effective treatment and prevention of many cancers may not be possible.

After exposure to a chemical carcinogen, the ultimate end-point is the development of cancer. This end-point does little by itself to give insight into how the chemical

might be carcinogenic. Although different carcinogens often produce tumors which consist of widely heterogenous cell types, and which manifest varying biological behavior, there is no known relationship between the nature of the chemical carcinogen and the nature of the induced neoplasm. Despite the enormous amount of time and material expended on cancer research, the genetic and biochemical basis for the altered properties of transformed cells is just beginning to be uncovered.

2. Stages of Carcinogenesis

The natural history of cancer development often involves a substantial portion of the life span of the host involved (i.e. months in rodents and many years in humans). During the intervening latent period, progressive tissue and cellular changes can be seen (108). The evolution from a normal cell through initiated, preneoplastic, neoplastic to malignant neoplasm has been divided into different stages. Studies, in the 1940's, of the induction of skin tumors with coal-tar in rabbits, and benzo[a]pyrene and croton oil in mice, led to the recognition of two distinct stages in chemical carcinogenesis, those of initiation and promotion (108-111). Subsequent work has expanded the concept to a multistage process. Within experimental systems, at least three different stages can be separated during the process of carcinogenesis. These stages are initiation, promotion, and progression.

a. Initiation

The initiation stage refers to the earliest event in chemical carcinogenesis. A brief exposure of a cell to an initiating agent can result in cancer development without the need for the presence of that carcinogen again (112). The initiated cell is not considered a cancer cell, but is able to respond differentially to varying types of stimulation which produce a focal proliferation (113). A cancer cell, on the other hand, has some degree of autonomy and can proliferate without the need for any known stimulation (113). The presence of the initiated state cannot be detected as such, and is evident only when some promoting or selecting environment results in a focal proliferation.

A tremendous amount of evidence supports the importance of some type of DNA alteration in the initiation process. Many of the classic genotoxic carcinogens have provided examples for this DNA specificity. Since the 1960's, it has been recognized that the metabolic conversion of many of the known carcinogens to highly reactive metabolites was required for their carcinogenicity. Several different types of carcinogens have been shown to generate reactive electrophiles, positively charged molecules which are capable of reacting with and altering electron dense cellular structures, like DNA (113). Induction of altered bases, miscoding lesions, and mutation have all been shown to occur early after exposure to certain genotoxic carcino-

genic chemicals (114). DNA is not the only possible target for a reactive electrophile. RNA, proteins, glutathione, and polysaccharides are all possible cellular targets (113).

Direct DNA damage, however, cannot explain how all of the chemical carcinogens induce neoplasia. Many carcinogens have been identified that have no measurable immediate interaction with DNA. These include several of the hypolipidemic agents and peroxisomal proliferators (94,95). . The activation and alteration of gene expression through various indirect routes has been suggested as another method for initiation of carcinogenesis (115,116). A decreased level of DNA methylation is one such indirect mechanism which has recently been proposed (117,118). Hypomethylation at the 5-position of cytosine has been shown to result in the induction of various gene functions (119,120). The 5-methylcytidine analogue, 5-azacytidine, which is incorporated into DNA but cannot be methylated, activates various genes and is reported to induce tumors in many tissues in rats (119,121). Several nongenotoxic carcinogens have been shown to induce hypomethylation of DNA (117,118).

The process of cell proliferation is an important aspect of the initiation process. Evidence indicates that there is a requirement for an early round of cell proliferation to be coupled with the exposure to a chemical

carcinogen for initiation and the induction of cancer to occur (122,123). For example, benzo[a]pyrene and dimethylbenzo[a]anthracene can be activated by the liver to form DNA adducts without initiating liver cancer, but if coupled with one round of cell proliferation, initiation and liver cancer does result (123). While the dependence of initiation on proliferation is well established in the liver, it also appears important in many organs, including urinary bladder and pancreas (124,125).

The mechanistic role of cell proliferation in initiation is not understood. One hypothesis is that the replication of DNA during cell proliferation "fixes" or makes permanent some genetic change induced by the carcinogen (111). It appears that the process of initiation is also multistaged, consisting of at least two steps, a biochemical alteration followed by cellular proliferation to "fix" the change.

b. Promotion

Promotion is the process in which the initiated cells are stimulated to become foci, nodules, papillomas, or polyps, which may then become the origin for the ultimate development of cancer (111). Unlike the initiated cell, the promoted cells can be detected by various biochemical and morphologic properties. In the liver the nodules differ from normal hepatocytes in cellular arrangement, blood supply, cytoplasmic and nuclear appearance,

microsomal enzyme activity, glutathione content, nistochemical staining characteristics, resistance to necrotizing effects of carcinogens, and in many other aspects (111,126-128).

Although the promoted cells are identifiable, precisely how the promoter or promoting stimulus selectively favors the proliferation of initiated cells over the surrounding ones is not known. Phorbol esters, the active portions of the classic promoter, croton oil, have been shown to produce many biochemical, metabolic, and biological effects in exposed tissues (112).

These effects include changes in morphology, microtubule polymerization, cell proliferation, enzyme induction, polyamine synthesis, phospholipid synthesis, membrane structure and function, and the release of prostaglandins. Other known promoting agents, such as phenobarbital,also nave an ability to cause a wide variety of tissue changes (129,130). No single effect or group of effects by known promoters have been identified as a prerequisite for their promoting ability.

The induction of ornithine decarboxylase (ODC) has been associated with tumor promotion in skin and the liver (131,132). The first and rate-limiting enzyme involved in polyamine biosynthesis is ODC. Polyamines are low molecular weight, cationic substances that interact with nucleic acids and support the synthesis of RNA, DNA,

proteins, and phospholipids (133). Physiologic concentrations of polyamines can stimulate translation, transcription, and replication processess in vitro (134). The induction of ODC acitivity has been shown to be a very early event in the process of cell proliferation (135). Partial hepatectomy, hormones, phenobarbital, and various other drugs which stimulate cellular proliferation have been shown to increase ODC activity (136,137). Several investigators believe that induction of ODC activity may be useful as an enzymatic marker for tumor promoters.

The focal proliferation of cells which results from the process of promotion has at least two outcomes. The cells may undergo remodeling or they may continue on to cancer. Evidence from rodent hepatic carcinogenesis systems, indicates that the vast majority (>95%) of the foci do eventually remodel to normal appearing cells (138,139). The few persistant foci show progressive loss of growth control over an extended period of time during which malignant neoplastic cells eventually may evolve (140).

c. Progression

The third stage of carcinogenesis is that of progression. Progression is the stepwise process through which the expanded or promoted initiated cell evolves into a cancer. The separation between the stages of promotion and progression is far from clear. It is clear, however, that at some point, cells within the persistant foci, which

results from the initiation and promotion processes, develop a degree of autonomy which allows them to grow without the need for external stimulation. Once this autonomy is attained, the neoplastic cells continue to progress toward a more malignant state. This self-generating process has been demonstrated in the liver and in other organs as well (111,112).

3. Chemical Carcinogenesis and Oncogenes

A model for the process of neoplastic transformation, which makes a cellular oncogene the key feature, has been proposed (141). According to this model, such an oncogene derives from a normally innocuous cellular DNA sequence which upon activation during carcinogenesis results in transformation of the affected cell. The original evidence for this model came from tumor virology.

The retroviruses are the only class of oncogenic viruses that have not evolved specific viral genetic sequences that are used to transform virus-infected cells. Retroviruses induce transformation by using normally present cellular genetic sequences (proto-oncogenes), which when incorporated into the viral genome, confer a transforming potential on that viral genome. Once the cellular gene is incorporated into the viral genome, it is freed from normal cellular control mechanisms and is regulated by the virus. Once allied with the viral genome, the cellular gene also acquires the genetic mobility normally associated with the

infectious virus. This relationship between retrovirus-associated oncogenes and normal cellular genes has been most thoroughly documented with the avian (Rous) sarcoma virus (ASV) (142). The transforming gene of ASV has been termed src and encodes a specific protein (142). The DNAs of normal, uninfected cells carry genetic sequences that appear identical to the viral src gene (143). In addition, a homolog of the src protein is present in normal, uninfected cells (144,145). At least ten other oncogenic retroviruses have been shown to use cellular genes as transforming agents (146). Each of these different retroviruses carry a different cellular gene generically termed v-one genes. Besides nucleic acid sequence homology studies, evidence for the presence of normal cellular genes within the viral genome have come from studies of ASV mutants. ASV mutants that have had their onc genes deleted can regain both the onc sequence and oncogenic potency by passing through normal cells (147). Recombination between the deleted ASV genome and the onc sequence, normally present in the cell, accounts for this restoration of function (148).

Besides providing proof of the existence of cellular onc genes, the retrovirus studies prompted the search for the presence of oncogenes in cells transformed by nonviral agents. The technique of gene transfer or "transfection" has been instrumental in the detection of

transforming genes in both viral and nonviral induced tumors. When DNAs of 3-methylcholanthrene-transformed mouse fibroblasts are introduced into untransformed, cultured fibroblasts, the recipient cells are transformed. The use of DNA from normal donor cells doesn't induce transformation in the recipient cell culture (149). These results provide direct proof that the DNA of a chemically transformed cell is altered in comparison to the DNA of an untransformed cell. Rat mammary carcinomas induced by N-nitroso-N-methylurea, mouse squamous-cell skin tumors induced with 7,12-dimethylbenz[a]anthracene, and chemically transformed guinea pig cells have all been shown to contain transferrable onc transforming genes (150-152). Several spontaneous tumors of unknown etiology, including several human cancers, have also been shown to carry transforming genes (153,154).

When the DNA sequences involved in 3-methylcholanthrene-induced fibroblast transformation from four separate transformants were compared, it was revealed that the same cellular gene was activated in each case (155). Analysis of the transforming genes in spontaneously occurring human colon carcinoma, bladder carcinoma, and a leukemia cell line indicates three different structures for these three genes (153). Further research is required to determine if oncogenesis within a given tissue or cell type leads to the activation of a specific gene.

It must be pointed out that most types of chemically induced and spontaneous tumors of both human and animal origin are inactive upon transfection (154,156). The hypothesis that *oncogene* activation represents a universal theme for both viral and nonviral-induced carcinogenesis remains to be validated by further work. It is clear, however, that for a number of different cancers, including several that are chemically induced, an identifiable cellular oncogene is involved in the neoplastic process.

4. Classification of Chemical Carcinogens

The chemicals which cause cancer comprise a large and structurally diverse group of organic and inorganic compounds. More than 30 different naturally occurring organic carcinogens have been identified. These include fungal metabolites such as aflatoxins and plant products such as pyrrolizidine alkaloids. The largest class of chemical carcinogens are synthesized or man made compounds. The majority of known organic chemicals are synthetic laboratory compounds. Over 700 chemicals are classified as animal carcinogens, only about 30 have been unequivocally associated with cancer induction in humans (157,158). Various descriptive terms and systems have been used for classifying chemical carcinogens.

It was first demonstrated by the Millers that most chemical carcinogens require metabolic activation to express their carcinogenic potential (159). With this in

mind the Millers introduced the terms precarcinogen, proximate carcinogen, and ultimate carcinogen to describe chemical carcinogens (160). These terms refer to the initial inactive compound, it's more active metabolites, and the metabolite actually responsible for carcinogenesis, respectively. The existence of a highly reactive electrophilic molecule, the ultimate carcinogen, following the metabolism of many chemical carcinogens has been repeatedly demonstrated. Because the vast majority of chemical carcinogens appear to initiate carcinogenesis through direct covalent interaction with DNA, the Millers descriptive classification is still useful. There are however, many examples of chemicals which apparently do not interact directly with DNA and still induce neoplasia.

The multistage models of carcinogenesis gave rise to another classification for chemical carcinogens. Chemicals which are capable of initiating neoplasia are called "initiators". These initiators are either "complete" carcinogens or "pure" initiators. A complete carcinogen has the potential for both initiating and promoting carcinogenesis, which is common for most chemical carcinogens. The pure initiator has no promoting potential of its own and requires the subsequent development of a promoting environment to produce cancer. Pure initiators may not exist, only urethane (in the mouse skin model) appears to fit the definition of a pure initiator

(110,161). Promoters are considered to be noncarcinogenic chemicals but when given after a low dose of an initiator, increase the number of tumors and decrease the latent period for tumor appearance (110). The phorbol esters in mouse skin and phenobarbital in rodent liver are examples of chemicals which are considered promoters (110).

There are many problems with the initiator/promoter classification system. Promoters are defined as noncarcinogenic because they are unable to induce cancer without the help of an initiator. This definition appears valid only within the narrow context of certain defined experimental protocols. Consequently, many investigators consider promoters to be carcinogens. Phenobarbital, when chronically administered to rodents, without exposure to any known initiator, results in increased hepatic tumors (162). Similarly, phorbol esters, when administered alone, can increase the incidence of skin tumors. Therefore, it is presently impossible to distinquish between pure promoting activity and weak carcinogenic potential of any given chemical. Using similar logic, the pure initiator should be considered noncarcinogenic because by definition it is incapable of inducing cancer without the addition of a promoter or promoting environment. Because of these inconsistencies and the lack of clear distinctions between the actual carcinogenic potentials of most initiators and promoters it is not universally accepted.

It has been proposed that chemical carcinogens be classified into different categories according to their ability to modify DNA or produce genetic damage (163). Genotoxic carcinogens are defined as those that are capable of producing DNA damage through formation of covalent bonds and therefore, correspond to carcinogens that act as electrophilic reactants (164,165). Epigenetic carcinogens are defined as those for which there is no evidence for DNA damage but rather act by indirect mechanisms (164,165). Promoters, hypolipidemics, peroxisomal proliferators, and all other nongenotoxic chemical carcinogens are classified as epigenetic carcinogens. This classification system has the advantage that there are short-term tests available, such as mutagenicity assays in bacterial or mammalian cells, which are useful in identifying genotoxic agents. Therefore, all chemical carcinogens which test negative for genotoxicity can be considered epigenetic agents. Though not universally accepted, this system of classification is now commonly used.

At present the descriptive terms for chemical carcinogens already mentioned, as well as others, are used when considered appropriate by a given investigator. A working knowledge of what these terms most commonly mean and special attention to the context in which they are used should be adequate for the comprehension of carcinogenesis. The "perfect" classification system for chemical

carcinogens awaits, as does the "perfect" cancer treatment, for more detailed knowledge concerning the biological basis for carcinogenesis.

D. Hepatic Chemical Carcinogenesis

1. Background

The use of liver carcinogenesis in experimental systems dates back to the 1930s when hepatocellular carcinomas were successfully induced with a variety of chemicals (165). The liver is the site for much of the metabolism of exogenous xenobiotics which enter the body. As previously mentioned, chemically-induced hepatic cancer has been shown to conform to the classic multistage theory of carcinogenesis. Hepatocellular carcinomas, induced by chemical carcinogens, are preceded by focal and hyperplastic lesions in the liver (167). Extensive research has been directed during the past 20 years to the process of hepatic carcinogenesis and its progression from initiated hepatocytes to hepatocellular carcinomas. These data makes experimental manipulation of rodent liver carcinogenesis currently one of the most used model systems in chemical-induced cancer research.

2. Stages in Development of Hepatic Carcinomas

The importance of the "hyperplastic" nodule as a possible precursor to hepatocellular carcinoma was first stressed in the late 1950s (168). Further studies also emphasized the association between hyperplastic nodules and

chemically-induced hepatocellular carcinomas (169). However, Peraino was the first to demonstrate initiation and promotion during the process of hepatocarcinogenesis. Peraino showed that the incidence of tumors in rats initiated with a short oral exposure to 2-acetylaminofluorene, a genotoxic carcinogen, could be promoted to 100% by chronic dietary exposure to 0.05% phenobarbital (170). Subsequently, numerous chemicals have been shown to be effective promoting agents of hepatic cancer when administered following acute, low level exposure to genotoxic carcinogens.

The observation that treatment of rats with hepatocarcinogenic nitrosamines induces focal populations of hepatocytes which are deficient in glucose-6-phosphatase activity, proved to be of great importance for morphological studies of hepatic carcinogenesis (171). Subsequently, it has been shown that phenotypically altered foci appear in the liver following treatment with various carcinogens. Other examples of aberrant phenotypes present within these foci include deficiencies of ATPase, acid or alkaline nucleases, serine dehydratase, and glycogen phosphorylase; increased expression of alpha-fetoprotein and gamma-glutamyl-transferase; and a lack of iron accumulation (172-179). These observations have become the basis of histochemical demonstration of enzyme-altered foci

(EAF) within the liver. Evidence that these EAF are actually preneoplastic expressions of initiated hepatocytes has come from different areas of research.

At least six models of chemically-induced hepatocarcinogenesis have been described (111,167,180). Each of these models consistently induce early hyperplastic nodules and EAF. The histologic and cytologic appearances of the hepatocytes within these focal areas are indistinguishable in the different models. In addition, this same degree of similarity exists at the biochemical level. Levels of microsomal P-450 and several other phase I enzymes are consistently low within hepatocyte nodules, while several microsomal phase II and cytosolic enzymes show a consistent elevation in activity (108,181). Gamma-glutamyl-transferase is greatly increased within the nodules or EAF (182,184). The histochemical staining characteristics of EAF induced by different chemical carcinogens are also remarkably similar (180). Recently a polypeptide of 21,000 molecular weight has been found in all nodules of the six different models as well as in hepatomas (185,186). This protein is not found in normal adult or fetal liver, in regenerating liver, or in normal hepatocytes after exposure to known carcinogens or

phenobarbital. Therefore, regardless of the type of chemical used as the initiating carcinogen or promoting environment involved, an early, detectable foci of altered hepatocytes is a common step.

Several studies have shown a good correlation between the numbers of EAF induced and the eventual number of tumors seen (109,111,112). Also, the nodules have been found to be a site of origin for cancer development using five different carcinogens in different models (111). Following a single chemically-initiated hepatocyte through its complete development to a hepatocellular tumor, currently technically impossible, would provide direct proof that nodules and EAF represent a preneoplastic, intermediate stage in the process of hepatocarcinogenesis. Despite the lack of such evidence, current data overwhelmingly supports the widely accepted conclusion that the hepatic nodules and EAF are focal populations of preneoplastic cells. The high regard for the validity of this conclusion has been reflected in the development of <u>in vivo</u> hepatic models, established on the basis of histochemical demonstration of EAF, for testing the carcinogenic potential of chemicals (187).

3. <u>Hepatic Initiation/Promotion Model Systems</u>

After Peraino developed a hepatic model in which initiation is brought about by a single or brief exposure to a carcinogen and promotion by long term exposure to

dietary phenobarbital (170), many investigators began using and modifying the model. Over the past 10 to 15 years, a better understanding of the dynamics of the basic in vivo hepatic initiation/promotion model has led to various modifications which have greatly increased the utility of the system. The importance of cell proliferation in the initiation stage of carcinogenesis was first demonstrated in the liver (122). It has been shown that by providing a stimulus for cell proliferation at the same time as exposure to the initiating carcinogen, the efficiency of the initiator is greatly increased as are the numbers of induced EAF. Proliferation, secondary to necrotizing doses of a chemical, such as CCL_4 or high doses of the chemical initiator, dietary deficiencies, or partial hepatectomy are all methods which have been used in various hepatic models (189). The use of the two-thirds partial hepatectomy is currently the most common method of providing a stimulus for cell growth during the initiation process in experimental hepatic carcinogenesis.

It is known that cell proliferation is also an essential phenomenon during promotion (111,112). The promoting environment apparently encourages the carcinogen-induced initiated cells to grow and produce focal proliferations. While the mechanisms by which promoting agents act are not known, most of the effective hepatic promoters, such as DDT and phenobarbital, induce

hypertrophy, hyperplasia, and cause hepatomegally after chronic exposure (165). Phenobarbital is commonly used as the promoting agent in hepatic initiation/promotion model systems.

There is clear evidence that carcinogens exert an inhibitory effect on cell proliferation in many tissues including liver (189). The discovery that one phenotype of carcinogen-initiated hepatocytes had acquired a resistance to this growth inhibition by carcinogens resulted in alternate methods of hepatic promotion. The resistant hepatocyte model of hepatic carcinogenesis incorporates the inhibitory effect of a carcinogen along with a stimulus for cell proliferation during the promotion process to select for the resistant, initiated hepatocytes (187,190). The advantages of the resistant hepatocyte model are the very rapid and synchronous development of the nodules, which is useful for studying the progression of the nodules to cancer (111,188). The use of 2-AAF during the promotion period, however, makes interpretation of results when testing for potential carcinogens, more complicated than with some other models.

An important aspect of all the in vivo hepatic initiation/promotion model systems is the separation between the initiation and promotion stages. While phenobarbital and DDT are potent promoters of experimental hepatic carcinogenesis, when administered at the same time

as known carcinogens they actually decrease the number of hepatic tumors (191,192). This effect appears to be directly related to the influence of these monooxygenase inducers on the metabolic activation or inactivation of the carcinogen. Effects like this and other possible confounding interactions between the initiating carcinogen and the chemical promoter or promoting environment, necessitate the inclusion of a latent period of one to two weeks between the end of initiation and the beginning of promotion. Evidence suggests that once the cells are initiated, the length of any delay until promotion is begun can be quite variable without altering the numbers of EAF or incidence of cancers produced (193).

Scherer and Emmelot administered single small doses of diethylnitrosamine (DEN) to partially hepatectomized rats and followed hepatic EAF with a histochemical stain for ATPase (194,195). They found that doses of less than 30 mg/kg of DEN, given by oral gavage, induced only a few EAF in the liver and no tumors, with the number of EAF being proportional to the dose of DEN. At higher doses, the proportionality was lost and hepatocellular carcinomas appeared as well. Pitot (178) extended this work by showing that promotion with 0.05% phenobarbital in the diet, following initiation with 5 or 10 mg/kg of DEN, given hours after partial hepatectomy, resulted in a 5-fold increase in EAF and the production of some hepatocellular

carcinomas. As shown earlier, by Scherer and Emmelot, these low doses of DEN produced no hepatic carcinomas for at least 24 months in treated rats (194,195). Also, only those animals which were promoted with phenobarbital exhibited hepatocellular carcinomas (178). Therefore, Pitots' protocol clearly distinquished between the initiation and promotion stages in experimental hepatic carcinogenesis.

Because the hepatic initiation/promotion model system described by Pitot is straight forward in design, variations of it have been used extensively to screen chemicals for hepatic initiating or promoting potential (189,196) The hepatic initiating and/or promoting potential of the chemical can be assessed by substituting the test chemical for either the known initiator, DEN, or the known promoter, phenobarbital.

The in vivo, short-term, initiation/promotion model system has become an important and widely used tool in the study of experimental chemical-induced hepatic carcinogenesis. Numerous researchers have shown that many of the hepatic initiation/promotion models can be used as sensitive screening assays for hepatocarcinogens as well as some non-liver carcinogens (189,197,198). Studies of the

natural progression and fate of EAF produced in these model systems continue to support the validity for their use as sensitive indicators of carcinogenic potential (193,199,200).

4. Phenotypic Properties of Rat Liver EAF

Foci of altered hepatocytes are regularly seen in the experimental induction of liver cancer in rats prior to the appearance of hepatocarcinomas. Since the fundamental work of Sasaki and Yoshida (166), the morphological characteristics of these focal and nodular lesions have been repeatedly described using conventional histopathological methods (126). The discovery that phenotypic differences of the altered hepatocytes correlated with the degree of expression of various cellular enzymes greatly facilitated identification and study of carcinogen-induced, putative preneoplastic foci. Foci of hepatocytes which were deficient in glucose-6-phosphatase (G-6-Pase) activity were first described about 1970 (171). Since then, numerous phenotypic alterations in the expression of cellular enzymes have been associated with altered hepatic foci (172-177). Specific histochemical staining techniques for marker enzymes allow rapid and accurate identification of carcinogen-induced EAF. Histochemically identifiable EAF appear earlier in experimental hepatocarcinogenesis than nodules, which make the EAF very useful in the in vivo, short term, hepatic carcinogenesis assays (126,127).

The most widely used enzymatic markers for EAF include the deficiency of G-6-Pase activity, the deficiency of membrane-bound ATPase activity, and increased gamma-glutamyl-transpeptidase (GGT) activity (109,127,201). The consistency and specificity of G-6-Pase, ATPase, and GGT as marker enzymes for EAF, in experimental rat hepatic carcinogenesis, have been compared by several different investigators (178,201-204). The least prevalent of the three enzymatic markers within EAF is the loss of G-6-Pase (203,204). Deficiency of G-6-Pase is almost always associated with either increased GGT or a deficiency of ATPase as well (203). Therefore, G-6-Pase deficiency is not the best choice as a marker for EAF in rat liver. Approximately 90 to 95% of the EAF produced in various rat liver experimental carcinogenesis models are GGT positive (201,205). While about 70% of the EAF are ATPase negative (201,202).

The resistance of hepatic nodules and EAF to the accumulation of iron is another commonly used phenotypic marker (127,206). This resistance to iron uptake has been shown to be nearly as consistent a marker as GGT in several hepatic models (127). However, resistance to iron loading is more unstable than GGT as a phenotypic marker. Without continued promotion, the altered hepatocytes lose the ability to resist iron quite rapidly, although, with the resumption of promotion, resistance to iron loading is

regained (206). An increase in GGT activity has been shown to be the most consistent and specific marker for chemical-induced hepatic EAF in the rat. Consequently, GGT activity is the histochemical marker most often used to study the progression of EAF and nodules or to assess the initiation/promotion potential of suspected hepatocarcinogens within the various rat liver models (109,165,193,201).

Using various hepatic models and histochemical markers, several researchers have reported that most of the EAF induced are reversible or disappear when the carcinogenic or promoter stimulus is removed (207-209). The GGT-positive EAF have been thoroughly studied within the context of the rat hepatic model in which a 70% partial hepatectomy and oral DEN (10 mg/kg) are used for initiation followed by phenobarbital promotion. The GGT-positive EAF induced by this regimen were shown to be stable whether or not the rats were continually promoted with phenobarbital (193). This same study suggests that a threshold dose level exists for promotion by phenobarbital. At dose levels below 0.0001% in the diet, chronic phenobarbital exposure failed to stimulate an increase in EAF compared to the control animals not promoted with phenobarbital. At dietary levels between 0.001% and 0.05%, chronic phenobarbital

administration resulted in a linear increase in the number of observed GGT-positive EAF. No further increases in the number of EAF resulted from phenobarbital levels above 0.05% in the diet.

III. MATERIALS AND METHODS

A. Chemicals

The major chemicals used in this study were trichloroacetic acid (Aldrich, 99+% reagent grade, Cat. No. 25,139-9), diethylnitrosamine (Sigma, reagent grade, No. 0258), phenobarbital (Baker, Cat. No. 2852), sodium hydroxide (Baker, reagent grade, Cat. No. 3722), tris(hydroxymethyl)aminomethane (Sigma, reagent grade, No. T-1503), tris(hydroxymethyl)aminomethane hydrochloride (Sigma, reagent grade, No. T-3253), sucrose (Baker, reagent grade, Cat. No. 4072), octyl phenoxy polyethoxyethanol (Sigma, No. T-6878), and N(gamma-L-glutamyl)-4-methoxy-2-naphthylamide (Polysciences, Cat. No. 2410). Other reagents were purchased from Sigma Chemical Co. or VWR Scientific.

B. Biological Reagents

Biological reagents used in this study were [1-^{14}C]palmitoyl-CoA (New England Nuclear, Cat. No. NEC-555), DL[1-^{14}C]ornithine (New England Nuclear, Cat. No. NEC-469),

bovine serum albumin (Sigma, No. A-4378), flavin adenine dinucleotide disodium salt (Sigma, No. F-6625), nicotinamide adenine dinucleotide (Sigma, No. N-7129), and coenzyme-A lithium salt (Sigma, grade III-L, No. C-3019).

C. Experimental Animals

Male, 5-6 week old, outbred Sprague-Dawley (S/D) rats were obtained from Washington State University, Department of Laboratory Animal Resources. These rats were chosen because: (1) the S/D rat is reported to have a low background level of GGT-positive EAF within control groups in the hepatic model involving partial hepatectomy, DEN, and phenobarbital promotion (210); (2) the male rat has been shown to be the most sensitive rodent model to the hepatocarcinogenic effects of various hypolipidemic peroxisomal inducers (211); and (3) the S/D rat has extensive historical use in hepatic toxicological and, specifically, carcinogenicity studies.

The rats were housed in groups of four in stainless steel wire bottom cages and provided feed (Ralston Purina Co.) and water ad libitum throughout the study. The drinking water was deionized and provided in clear glass water bottles (16 oz) with rubber stoppers and stainless steel controlled-flow siper tubes. The animal rooms were

maintained on a 12-hour on/off automatic light cycle and temperature was controlled within a range of 68-73 F. All animals were given at least a 5 day acclimatization period prior to placing on experiment.

D. Specific Chemicals, Routes of Exposure, and Dosages

1. Trichloroacetic Acid (TCA)

Exposure to TCA, as the sodium salt, was provided via the drinking water in order to best simulate the route of environmental exposure. The selection of dosages were based on the 50 mg/kg minimum dose of TCA required for hepatic peroxisomal induction in the rat as reported by Elcombe (49). Based on average daily water consumption, a concentration of approximately 500 ppm in drinking water is required to obtain a 50 mg/kg/day dose in a rat. Therefore, the 500 ppm concentration was chosen as the middle dose and a factor of ten used to derive the high and low doses, 5000 ppm and 50 ppm, respectively.

Because a highly pure commercial source of the TCA sodium salt is not available, the drinking water for the administration of TCA was prepared as follows: TCA (250g of 99+% reagent grade) was added to 1 liter of distilled water. Sufficient reagent grade sodium hydroxide was added to bring the solution to a pH between 6.5 and 8.0. This solution was then brought to a 50 liter volume to obtain the final 5000 ppm concentration. The 50 ppm and 500 ppm concentrations were prepared by dilution of the 5000 ppm

concentrate in distilled water. All TCA solutions were prepared weekly and the watering bottles were changed twice weekly.

Animals which received a single dose of TCA were given 1500 mg/kg TCA via intragastric intubation in a maximum total volume of 1.5 ml per rat.

2. Diethylnitrosamine (DEN)

DEN was diluted with distilled water to a final concentration of 2 mg/ml. The rats were given this DEN solution via intragastric intubation at a dose of 10 mg/kg with the maximum total volume not exceeding 1.5 ml.

3. Phenobarbital (PB)

Twenty-five grams of PB were weighed out and initially dissolved in 200 ml of weak sodium hydroxide solution. The resulting mixture was brought to 50 liters to obtain the final 500 ppm concentration. The PB drinking water was prepared fresh twice weekly and the water bottles were changed two times weekly.

E. Experimental Designs

1. Hepatic Initiation/Promotion Model System

a. Initiation Assessment

Figure 1 and Table 1 illustrate the design and protocol of the initiation study. There was a total of 7 animal groups (A-G) in the initiation portion of the study. All animals were 2/3 partial hepatectomized (PH) using the method of Higgins and Anderson (212), except for group F

which received a sham operation. The sham procedure was identical to the hepatectomy except no hepatic tissue was removed. The surgical procedures were followed 24 hours later by single-dose of DEN, 10 mg/kg, (group A) or TCA, 1500 mg/kg, (group B), given as an oral gavage. The remaining groups were administered TCA at 5000 ppm in their drinking water for 10, 20, or 30 days. Two weeks following the initiation period, all groups were administered PB (500 ppm) in the drinking water for the remainder of the study. Animals were randomly sampled 24 hours after the end of initiation, 24 hours prior to the start of promotion and at the 3, 6, and 12 month intervals of the promotion period. An additional experimental group (group H), the age-matched negative control, which received no experimental manipulation at all, was sampled only at the 12 month time period due to the limited number of animals available for inclusion into this group at the beginning of the study.

b. Promotion Assessment

Figure 2 and Table 2 illustrate the design of the promotion study. There were 7 experimental groups (M-S) in the promotion portion of the study. The experimental animals were partial hepatectomized or sham operated (group Q), followed 24 hours later by oral gavage with either DEN at 10 mg/kg body weight or distilled water (groups Q and R). Two weeks later, 500 ppm PB or TCA at 50, 500, or 5000

ppm were added to the drinking water of all groups except R. Animals were randomly sampled at the 2 week, 1, 3, 6, and 12 month intervals of the promotion period. An additional experimental group (group T), age-matched negative control, was sampled only at the 12 month interval.

c. Quantification of Enzyme Altered Foci (EAF)

Animals were killed by CO_2 asphyxiation and the livers were quickly removed and weighed. Cross sectioned samples were taken from the right lateral anterior and posterior lobes. These liver sections were then immediately frozen in liquid nitrogen and stored at -70C. Five-micron sections were cut on a cryostat, placed on a slide, air dried, and fixed in cold absolute ethanol for 30 minutes. Staining for GGT activity was done by the histochemical techniques of Rutenburg et al. (213) and counter stained with hematoxylin. Foci positive for GGT, containing 9 or more nuclei, were counted directly from the prepared slide using a HIPAD digitizing tablet, Houston Instruments, coupled optically to a microscope. The tissue area, number of foci, and foci area were all recorded. An area of liver of at least 3 cm^2 was evaluated for each animal.

d. Peroxisomal Beta-oxidation Assay

Fresh liver samples were collected at all sampling periods, frozen in liquid nitrogen, and stored at -70C. These samples were weighed frozen, then placed in 10

volumes of 0.25 M sucrose solution on ice. The samples were homogenized using a Brinkmann Polytron Homogenizer, with 3-15 second bursts at high speed. The ability of the homogenates to oxidize palmitoyl-CoA was measured directly by the oxidation of [1-^{14}C]palmitoyl-CoA by the method of Lazarow (86). The conversion of acid-insoluble [^{14}C]palmitoyl CoA to acid-soluble [^{14}C]acetyl CoA was measured by liquid scintillation spectrometry.

e. Necropsy and Histopathology

The experimental animals were weighed prior to treatment and at the time of sacrifice. The liver, kidneys, spleen, and thymus were weighed at necropsy and organ-to-body weight ratios were calculated. Complete necropsies were performed on all animals. All gross lesions or tumors were recorded. The brain, heart, lungs, kidneys, spleen, thymus, pancreas, adrenals, testes, lymph nodes, gastrointestinal tract, urinary bladder, muscle, and skin were fixed in 10% buffered formalin, processed, and stained with hematoxylin and eosin (H and E) prior to examination by light microscopy. Microscopic examination of livers was done on ethanol fixed, H and E stained, frozen sections taken from the same liver samples used for evaluation of GGT-positive foci.

2. Subchronic Toxicological Evaluation

Four experimental groups of 10 animals each received 0, 50, 500, or 5000 ppm TCA in the drinking water for 90 days.

Measurement of hepatic mixed function oxidase, peroxisomal beta-oxidation, serochemistries, and organ-body weights; as well as complete necropsies and histopathology were all performed at the termination of the study. The peroxisomal beta-oxidation assay, organ-body weights, necropsies, and histopathology were performed as previously described.

a. Serochemistries

Half the animals in each group were lightly anesthetized with ether and bled via cardiac puncture at the beginning of the study. All the animals surviving at the end of the 90 day study were bled by cardiac puncture immediately after CO2 asphyxiation. A serum biochemical profile was done at Washington State University's Veterinary Clinical Diagnostic Laboratory on all samples. Tests included blood urea nitrogen (BUN), creatinine, calcium, phosphorus, total protein, albumin, glucose, cholesterol, alkaline phosphatase, creatine phosphokinase (CPK), alanine aminotransferase (ALT), and GGT.

b. Measurement of hepatic microsomal enzymes

Livers from the experimental animals were immediately placed in and washed with ice-cold physiological saline for the isolation of the "microsomal" fraction. Total cytochrome-P-450 was determined by the method of Omura and Sato (214). Microsomal cytochrome-P-450-dependent O-dealkylation of 7-ethoxycoumarin was measured with the

technique of Prough, Burke, and Mayer (215) and aminopyrine N-demethylase activity was measured using the assay developed by Werringloer (216).

3. Hepatic Ornithine Decarboxylase (ODC) Assay

A single intragastric gavage dose of 1500 mg/kg TCA in distilled water (total volume of 1 ml) was administered to each of 8 male Sprague-Dawley rats (130-150 grams), 8 control animals recieved distilled water. The rats were killed by CO2 asphyxiation either 5 or 18 hours post treatment (4 controls and 4 treated rats at each sampling). Fresh liver samples were immediately homogenized in ice-cold buffer using a Brinkman Polytron Homogenizer. Ornithine decarboxylase was assayed on 144,000g supernatants. The enzyme activity was determined by measuring the release of $^{14}CO2$ from DL-[1-^{14}C]ornithine as described by Bethell and Pegg (217) and adapted by Savage, et al (218).

4. Statistical Analysis

The data were evaluated with an analysis of variance (ANOVA) program from a Statistical Analysis System (SAS, Helwig and Council, 1979) package on the University of Idaho's IBM P3887 computer system. Statistical significance was determined by analysis of variance and least square means multiple comparisons. An alpha level of $P \leq 0.05$ was considered as a significant difference between groups.

IV. RESULTS

A. Induction of GGT-Positive Foci

1. Initiation Bioassay

The results of the GGT-positive foci initiation bioassay are summarized in Table 4. Only the positive control (group A) which had approximately 2, 10, and 9 foci/cm^2 at 3, 6, and 12 months, respectively, showed a statistically significant effect. The numbers of GGT-positive foci/cm^2, in group A, increased from 3 to 6 months, but, no increase over the 6 month level was seen at the 12 month sampling. The initiatior control (group G) had almost no GGT-positive foci when compared to age-matched negative control animals at 12 months. These results are consistent with those of other investigators, who have shown that both partial hepatectomy and phenobarbital promotion are necessary to optimize induction of DEN-initiated enzyme altered foci. The four TCA treatment groups (B, C, D, and E) failed to demonstrate significant induction of GGT-positive foci.

The mean area of the GGT-positive foci within each experimental group increased at each successive sampling period (Table 4). A similar time-dependent increasing pattern for size of areas as well as volumes of EAF have

been reported by other investigators. The mean area of the GGT-positive EAF for the positive control (group A) was consistently larger than those of other groups at all sampling intervals.

2. Promotion Bioassay

The results of the promotion experiment are summarized in Table 3. As with the initiation protocol results, the positive control (group M) had GGT-positive foci at a level significantly ($P \leq 0.05$) higher than that seen in the other groups at the 3, 6, and 12 month intervals. In contrast to the initiation positive control (group A), the promotion positive control (group M) showed an increase in numbers of GGT-positive EAF at each sampling period. The three TCA-promoted groups (N, O, and P) showed a similar pattern over the course of the experiment. The lack of significant foci induction within the promoter controls (group S) or initiator/promoter controls (group R) again supports the need for both partial hepatectomy and phenobarbital promotion to optimize induction of DEN-initiated foci.

The low dose (50 ppm) TCA-treated promotion animals (group N) had significantly ($P \leq 0.05$) greater foci induction at 3 months than any of the negative controls (groups Q, R, and S) except for group R. This same level of foci induction is seen with a high dose (5000 ppm) of TCA promotion (group P). The statistical differences between the low and high TCA dose groups (N and P) and control

group R were $P \leq 0.06$ and $P \leq 0.07$, respectively. Although, the level of GGT-positive foci induction seen at 3 months with 500 ppm TCA promotion (group O) was greater than all the negative controls, the increase was not statistically significant. However, at the 6 and 12 month intervals all three dose levels of TCA promotion (groups N, O, and P) resulted in statistically significant ($P \leq 0.05$) greater levels of foci induction when compared to any of the negative controls.

The mean GGT-positive foci areas increased in size at each sampling within all the experimental groups. The largest mean area was consistently seen in the positive control (group M), with the three TCA-promoted groups (N, O, and P) next largest, while the negative controls (groups Q, R, and S) and the age-matched control at 12 months had the smallest areas.

B. Hepatic Peroxisomal Beta-Oxidation Assay

A significant ($P \leq 0.05$) increase in peroxisomal specific palmitoyl-CoA oxidation was induced only in the high dose TCA (5000 ppm) groups (P and Q) in the promotion protocol (Table 5). This increase was consistently seen at all sampling periods. The magnitude of the increase over controls, 10-20%, was small when compared to those increases associated with "typical" peroxisomal proliferators, such as clofibrate.

The results of an experiment in which clofibrate (250 mg/kg) in corn oil, was administered daily, via gastric gavage, for 14 days, to male, 130-160 gram, WSU S/D rats are summarized in Table 6. The increase in peroxisomal specific palmitoyl-CoA oxidation induced by clofibrate, a known peroxisomal proliferator, was 5-6 times the activity seen in corn oil treated control animals. This increase in peroxisomal activity is consistent with what is reported in the literature for clofibrate and other known peroxisomal proliferators.

A significant ($P \leq 0.05$) depression of palmitoyl-CoA oxidation was evident at all sampling periods for the positive control (group M) and appeared to be related to PB treatment (Table 5). Similar PB effects on palmitoyl-CoA oxidation activity has been reported by other investigators.

No significant stimulation of palmitoyl-CoA oxidation was seen at any of the sampling intervals for the TCA treated initiation groups (Table 7).

C. Organ/Body Weights

No significant differences in body weights between experimental groups in the initiation or promotion protocols were seen at any of the sampling periods. No significant changes in spleen, liver, or kidney weights as a percentage of body weight were observed in TCA-treated rats, when compared to controls, at any of the sampling

times in either the initiation or promotion groups (Tables 8 and 9). This lack of effect on liver weight is compatible with the minor peroxisomal-associated palmitoyl-CoA stimulating effects produced by high dose TCA. Hepatomegaly has been shown to accompany the large increases in peroxisomal enzyme activity associated with known hepatic peroxisomal inducers. Treatment with clofibrate resulted in a significant ($P \leq 0.001$) increase in liver weight when compared to control animals (Table 6).

A significant ($P \leq 0.05$) increase in liver weight was detected in the positive control (group M) of the promotion groups through the 6 month sampling period (Table 8). This increase is consistent with hepatomegaly due to microsomal induction commonly seen with PB treatment. This same increase was not seen at 12 months, however.

D. Gross and Microscopic Pathology

No significant gross pathologic lesions could be attributed to TCA administation at any of the sampling periods. Approximately 25% of the animals that had been partial hepatectomized had small, 3-8 mm, well localized, abscesses present on the liver at the site of surgery. These abscesses commonly contained a thick white purulent exudate at the early sampling periods (3 months or less), while a dry brownish caseated exudate was most commonly seen at the later sampling intervals. Between 50 and 60% of the animals, both partial hepatectomized and sham

operated, had fibrinous adhesions between the surface of the liver and the ventral peritoneum and diaphragm. Microscopically, only an occasional hepatic abscess was seen.

The procedures required for this study resulted in an unavoidable delay in removal and processing of liver samples for histopathologic examination at each sampling interval. Therefore, a mild to moderate degree of autolysis was evident within all hepatic sections. This degree of autolysis had no effect on histochemical evaluation for GGT-positive EAF. However, microscopic evaluation for minor hepatocellular changes was compromised.

Mammary tumors developed in several animals in both the initiation and promotion groups between 9 and 12 months of the experiments. However, the incidence of these tumors was no greater within any of the experimental or control groups, (Table 10). The 2 to 7.5 cm (diameter) tumors developed subcutaneously, in a ventrolateral position between the axillary and inguinal areas. Most contained a central cavity filled with a dark brown viscous fluid. Microscopically the tumors were shown to be mammary cystic adenomas. Animals with mammary tumors were excluded from all assays at the 12 month period.

Only one hepatic tumor, in an animal from promotion group P at 12 months, was detected. The tumor was approximately 2 mm in diameter, GGT-positive, and was diagnosed microscopically as an hepatocellular carcinoma.

Histochemical staining for GGT yielded positive results not only for the EAF and normally positive bile duct epithelium, but also demonstrated larger areas of diffuse hepatocyte staining that radiated out from portal tracts. Unlike the GGT-positive foci, in which all the hepatocytes stained intensely, not all the hepatocytes within these larger areas were GGT-positive and the staining was much less intense. Within the initiation groups, all of which recieved PB in the drinking water, the areas of diffuse GGT-positive staining were seen in nearly all of the livers. The incidence of this diffuse staining was similar in all initiation groups, including the controls. In contrast, within the promotion groups only the positive control (group M), which also recieved PB, showed the same degree of diffuse staining. Within the remaining promotion groups similar areas of diffuse staining also developed but only in about 40-50% of the livers.

No significant or consistent microscopic lesions could be attributed to TCA administration at any of the sampling periods.

E. Subchronic (90 day) Assay

1. Hepatic Peroxisomal Beta-Oxidation Assay

The effects on hepatic peroxisomal beta-oxidation by 90 day exposure to TCA (50, 500, or 5000 ppm), in the drinking water, were similar to those produced in the promotion bioassay (Table 11). Only the high dose of TCA (5000 ppm) caused a significant ($P \leq 0.0005$) induction of hepatic peroxisomal beta-oxidative activity. Again, the magnitude of the increase in activity is relatively minor when compared to effects of known peroxisomal inducers.

2. Effects on Hepatic Microsomal Enzymes

No dose-dependent changes in total hepatic P-450 content were induced by TCA exposure, (Table 12). Aminopyrine N-demethylase activity was increased in a dose-dependent manner as shown in Table 13. This increase was not however, significant at the 95% confidence interval. The small degree of freedom used in the statistical analysis most likely contributed to the lack of significance. While livers from at least 6 different rats were used in both the aminopyrine N-demethylase and ethoxy-coumarin O-de ethylase assays, the liver samples were combined into one of two different common samples resulting in an n of 2. A significant difference between the control and high dose group (5000 ppm) was seen at the 90% confidence level.

A nonsignificant increase in ethoxy-coumarin O-de ethylase activity was seen in the the high dose group (Table 14). Again, the small degree of freedom resulting from the combining of the liver samples into two different common samples likely contributed to the lack of significance.

3. Organ/Body Weights

No significant differences in body weights or organ weights as a percentage of body weight were noted between the three experimental groups and controls, (Table 15).

4. Serochemistries

The data from the biochemical profile of serum samples are summarized in Table 16. The initial serological values, obtained immediately prior to the rats being placed on experiment, showed that all groups were comparable for the parameters measured.

After 90 days the serum cholestrol levels of the three TCA exposed groups (50, 500, or 5000 ppm) were significantly ($P \leq 0.05$) lower than the level of the control animals. A significant ($P \leq 0.05$) increase, approximately 50% over controls, in serum alanine aminotransferase (ALT) was seen in the high dose TCA (5000 ppm) animals, at 90 days. No other significant differences between experimental groups were noted.

5. Gross and Microscopic Pathology

Other than one control rat with unilateral hydronephrosis, no gross pathologic lesions were seen at necropsy. Microscopic examination of tissues was also unremarkable. No significant or consistent microscopic lesions could be contributed to TCA exposure. Again, an unavoidable mild to moderate level of autolysis within the liver sections made evaluation for minor hepatocellular damage difficult. However, no consistent hepatic lesions within the high TCA dose (5000 ppm) animals were seen which would account for the increase in serum ALT.

F. Hepatic Ornithine Decarboxylase (ODC) Assay

The results of the ODC assay are shown in Table 17. Five hours after TCA administration (1500 mg/kg) hepatic ODC activity was significantly ($P \leq 0.005$) elevated, a 69.5 fold increase, over control values. After 18 hours ODC activity was still significantly ($P \leq 0.001$) elevated.

G. Measurements of Drinking Water Intake

The consumption of drinking water, both treated and untreated, was monitored at 3 weeks, 3 months, and 6 months of the promotion protocol. The values shown in Table 18 represent the mean daily water intake from 4 animals in each group over a 5 day period. Measurements were made daily while the animals were housed in plastic metabolism cages, after 3 days acclimation. Both water consumption and urine output were measured.

The amount of water consumed per kg body weight declined with time within the TCA treated animals as well as in the nontreated rats. The decline in consumption of PB treated water, over time, was small compared to the other groups. The lowest water intake was consistently seen in the high dose TCA (5000 ppm) groups. The medium dose TCA (500 ppm) rats recieved a dose of TCA of about 50 mg/kg/day through 3 months. However, by 6 months the dose of TCA in the medium dose group was down to approximately 30 mg/kg/day. While the dose of TCA recieved by the high dose (5000 ppm) group also declined over time, the dose at 6 months was greater than 275 mg/kg/day.

V. DISCUSSION

Recent reports indicate TCA induces hepatic peroxisomal enzyme activities (48, 49). These reports, along with increased metabolic TCA formation in the mouse compared to the rat following trichloroethylene administration, have led several researchers to speculate that TCA levels may be important for explaining the carcinogenicity of trichloroethylene in the mouse (31, 32).

It was considered important for this research that the selection of the experimental doses of TCA reflect the possible link between hepatic peroxisomal effects and hepatic carcinogenesis. The minimum daily dose of TCA reported to cause hepatic peroxisomal induction, in the rat, is 50 mg/kg (49). This dose was used as the target

for the medium dose when calculating the concentration of TCA needed in the drinking water, with a factor of ten used to derive the high and low doses. Table 17 shows that, for the first 3 months, the medium concentration (500 ppm) of TCA in the drinking water achieved a daily intake of TCA very close to the target dose of 50 mg/kg. The normal decline in water intake per kg body weight which occurs with maturity in rats resulted in the lower daily dose of about 30 mg/kg at 6 months for the 500 ppm groups. The use of 5000 ppm TCA as the high dose ensured that some experimental animals remained well above the 50 mg/kg/day level throughout the study.

All three concentrations of TCA in the drinking water (50, 500, and 5000 ppm) used in this study greatly exceed the highest environmental levels, at several hundred ppb, of TCA found in chlorinated drinking water. (1, 2). However, high doses are used in most carcinogenicity and toxicological studies and are necessary to reduce to manageable levels the numbers of experimental animals needed.

The hypothesis which was tested by this research was that TCA is capable of inducing and/or modulating hepatic carcinogenesis in the rat. Chronic, life-span, exposure bioassays which quantitate actual tumor incidences are considered essential to provide the best evidence for the carcinogenic potential of a given chemical. The tremendous

expense and time required to complete a life-span carcinogenicity bioassay limit its usefulness. The development of short term, in vivo bioassays which are capable of assessing both the initiating and promoting potential of chemical carcinogens, have made the screening process of suspected carcinogens less expensive and more manageable.

Various hepatic initiation/promotion bioassays for testing chemicals for carcinogenic potential have been validated for use in the rat (189, 197-200). The in vivo, hepatic initiation/promotion bioassay which combines partial hepatectomy, DEN for initiation, and PB as a promoter, is among the best studied and widely used system (189, 194-196). It is widely accepted that EAF, induced in the hepatic initiation/promotion bioassays, are indicators of an early stage in the carcinogenic process. Therefore, quantification of the EAF is useful as an indicator of the initiating and/or promoting ability of the tested chemical (126, 127, 193, 201, 206).

Initiating potential. The results from the initiation protocol used in this study fail to provide any evidence for initiating activity by TCA in the rat hepatic bioassay. The data in Table 3 show that exposure of rats to a single large dose of TCA (1500 mg/kg) or to 5000 ppm in the drinking water for 10-30 days, followed by PB promotion, doesn't result in induction of GGT-positive EAF signficantly above levels in the negative control groups.

In contrast, the animals which recieved a partial hepatectomy and a known carcinogen (DEN) for initiation and PB promotion had a classic pattern of EAF induction very similar to that reported by other investigators (193).

Most known chemical carcinogens are genotoxic and are thought to initiate tumorigenesis through a direct action on DNA (113, 114, 159). Both the lack of initiating activity by TCA in this study and negative short-term, in vitro, mutagenicity tests (43, 44) provide evidence that TCA lacks significant genotoxic activity.

Promoting potential. The promoting activity of TCA was also invesitgated using the rat hepatic system. Table 4 shows that after 3 months of TCA administration via the drinking water, significant, though somewhat weak, promotion activity was observed for both the low dose (50 ppm) and high dose (5000 ppm). While the medium dose of TCA (500 ppm) was associated with more GGT-positive EAF than the negative controls the increase was not significant. By 6 months all three levels of TCA exposure resulted in significant increases in GGT-positive foci. Again, at 12 months TCA exposure was associated with a non-dose-dependent induction of EAF significantly above that seen in the negative control groups. These data indicate that TCA possesses significant promoting activity within this rat hepatic bioassay.

The promoting activity exhibited by TCA in this bioassay was not, however, of the magnitude seen with PB, a known potent hepatic tumor promoter. It has been reported that a threshold dose level exists for the promoting activity of PB (193). Data from this study showed no evidence that a threshold dose level or a dose-response relationship exists between the promoting activity and the concentrations of TCA used. If a threshold dose for the TCA promoting activity does exist, it must be at a level below the lowest concentration (50 ppm) used in this study.

Further evidence of the TCA promoting activity was seen in the sizes of the GGT-positive EAF. The mean areas of the induced EAF were, at each sampling period, consistently larger in the three TCA exposed groups when compared to the negative control groups. However, similarly to the data on the numbers of EAF, the mean areas of the foci in the three TCA test groups were smaller than those in the positive control group (PB promotion) at each sampling. Larger foci areas in promoted groups as well as a similar pattern of foci size increasing with time are consistent with results of others (109, 165, 193, 201).

The positive control group in the promotion protocol showed a pattern of significant EAF induction, at all three sampling periods, comparable to that seen in the identically treated positive controls from the initiation protocol.

Hepatic peroxisomal activity. Although TCA has been reported to cause hepatic peroxisomal stimulation in rats and mice (48, 49), TCA would not be considered a hepatic peroxisomal inducer based on the results from this study. Within the promotion protocol (Table 5) it was apparent that both groups which recieved the highest dose of TCA (groups P and Q) exhibit, at each sampling interval, a consistent increase in the ability to oxidize ^{14}C labeled palmitoyl-CoA. While this increase in peroxisomal-associated activity was significant at all five sampling times, it was not of the magnitude seen with exposure to known peroxisomal proliferators. The 90-day, subchronic exposure to TCA produced (Table 11) results similar to those of the promotion protocol. An increase, of approximately 15% over controls, in palmitoyl-CoA oxidative activity is seen only in the 5000 ppm treated rats.

Within the initiation protocol no increase in peroxisomal beta-oxidative activity was seen within any group at any sampling (Table 7). It would be expected that an increase in palmitoyl-CoA oxidation similar to that seen at the 5000 ppm level in the promotion study should appear in some of the TCA exposed groups at the first sampling period. The reported minimum dose of TCA needed to stimulate hepatic peroxisomal activity is 50 mg/kg/day for 10 days (49, personal communication with C.R. Elcombe). With exposure to 5000 ppm TCA in the drinking water for 10,

20 or 30 days (groups C,D,E,F) this minimum dose should have been easily obtained. The most obvious difference between the high dose promotion groups and the TCA treated initiation groups, at the first sampling, was the interval between the partial hepatectomy or sham surgery and the start of TCA administration, (Figures 1 and 2). Whether the surgical stress or some other factor(s) is responsible for this lack of early peroxisomal stimulation in the initiation protocol can not be answered without further experimentation.

Because TCA exposure did not result in the level of hepatic peroxisomal stimulation expected from known peroxisomal proliferators, several rats were exposed to clofibrate to serve as positive controls for the C^{14}-palmitoyl-CoA oxidation assay used in this study. The clofibrate exposure (Table 6) produced a large increase in peroxisomal-associated palmitoyl-CoA oxidation. This approximately 6 fold increase over controls is consistent with published reports (81, 82) for clofibrate and other known peroxisomal proliferators.

Hepatomegaly is another common effect associated with exposure to proxisomal proliferators (81). The clofibrate treated rats do show a significant increase in liver weight when compared to controls (Table 6). However, no significant increase in liver weights can be attributed to TCA

exposure within any experimental group from the initiation protocol, promotion protocol, or the 90-day study.

Although TCA shows the ability to stimulate (10-20% over controls) peroxisomal-associated, palmitoyl-CoA oxidation, data from this research indicates that this weak peroxisomal effect is unlikely to be related to TCA's promoting activity. TCA exhibits promoting activity at all three concentrations (50, 500, 5000 ppm) used in the promotion protocol. This is in contrast to the minimal peroxisomal effects which are seen only at the 5000 ppm level.

Induction of hepatic ornithine decarboxylase. A single large dose (1500 mg/kg) of TCA caused significant induction of ODC activity, which was apparent at both 5 and 18 hours after administration (Table 17). ODC induction has been associated with promotion of carcinogenesis in both the skin and liver (131, 132) and has been suggested as an enzymatic marker for tumor promotion (137). More recent work has raised questions about the necessity of ODC induction for hepatic promoting activity (219). Whether ODC induction can be used as a biochemical marker for tumor promoters is not currently known. However, it is known that inducton of ODC activity is closely related to the process of cell proliferation (135) and that several known hepatic tumor promoters, including phenobarbital, can stimulate ODC induction (137, 218).

Further research is needed to describe dose-response relationships and time course data on ODC induction by TCA. No relationship between TCA's relatively weak promoting activity and its stimulation of hepatic ODC activity can be shown from these data. It is clear however, that TCA shares an ability to induce hepatic ODC activity with other known hepatic promoters.

Induction of hepatic microsomal enzymes. This study produced no evidence that TCA exposure causes significant induction of hepatic microsomal enzymes. No significant increase in total hepatic P-450 content was apparent at any concentration of TCA used in this study (Table 12). There was a nonsignificant dose-related increase in aminopyrine N-demethylase activity over controls (Table 13) and a nonsignificant increase in ethoxy-coumarin O-de ethylase activity, within the 5000 ppm group (Table 14). As mentioned earlier, the lack of statistical significance is most likely due to the small degree of freedom resulting from the combining of individual samples. However, even if the differences were significant the size of the increases in activity were not very large.

Gross, microscopic and clinical pathology. There was a lack of gross and microscopic abnormalities which could be attributed to TCA exposure in any of these studies. The lack of tissue and organ damage indicated that exposure to TCA via the drinking water, at the levels used in this

research, was fairly innocuous in the rat model system. The most significant pathologic lesions noted in these experimental animals was the development of mammary tumors between the ninth and twelfth months of the initiation and promotion studies. There was however, no evidence of any relationship between the incidence of mammary tumors and treatment of any experimental group or groups.

The serological profiles from the 90-day studies appeared to support a lack of significant TCA toxicity (Table 16). A significant decrease in serum cholesterol was seen within all three TCA treatment groups when compared to the untreated controls. The reason for this lowered cholesterol is not immediately apparent. Whether or not this cholesterolemic effect can be correlated with any other biological effects associated with TCA exposure, cannot be answered without further research.

The other significant alteration in the serum chemistries was the increase in alanine aminotransferase (ALT) in the high dose group. Elevated serum ALT is considered fairly specific for hepatocellular damage in the rat. While minor hepatocellular damage from the high dose TCA exposure was suggested by the elevated ALT, it could not be either verified nor dismissed histopathologically. Subtle or minor histopathological damage to livers from any of the experimental animals would be very difficult to determine because of the low to moderate level of autolysis

present within the hepatic tissue sections. As mentioned previously, the procedures involved in collection and processing of the livers from the experimental animals made a low level of autolysis unavoidable. Serum GGT levels, which have been reported to increase in the presence of hepatic tumors (220), remained at low, undetectable levels in this study. No other meaningful changes in serum chemistries were seen.

Body and organ weights. Changes in body and organ weights are readily evaluated and commonly used as a measure of general toxicty within toxicological studies. The body and organ weight data from these studies provided more evidence for low toxicity associated with exposure to the levels of TCA used in this research. The only significant differences involving organ/body weights was an increase in liver weight, within the positive control group (M), seen at the 3 and 6 month sampling intervals (Table 9). This hepatomegally was consistent with chronic PB administration which was associated with induction of microsomal enzymes. No effects on body or organ weights could be attributed to TCA.

Summary. The speculation that metabolically determined differences in TCA levels between the mouse and the rat could explain trichloroethylene (TCE) hepatic carcinogenicity in the mouse and not the rat (31, 32) is based on the reported hepatic peroxisomal proliferating activity of TCA

(48, 49). The results of this research indicate that TCA possesses tumor promoting activity in the rat liver. However, the evidence provided no correlation between the promoting ability of TCA and its very weak stimulation of hepatic peroxisomal beta-oxidation.

The promoting activity of TCA, though not as potent as phenobarbital, could help explain why TCE-induced hepatic tumors appear in the mouse and not the rat. Phenobarbital, a model hepatic tumor promoter, has been shown to produce increases in the incidence of hepatic cancer without any known exposure to an initiating agent (162). It has been suggested that epigenetic, tumor promoters produce liver tumors by promotion of endogenous preneoplastic lesions (199). The increased metabolic conversion of TCE to TCA in the mouse compared to the rat (31, 32) could result in promotion of endogenous initiated cells in the mouse and the development of hepatic tumors. The validation of any relationship between TCA- and TCE-induced hepatic tumors in rodent models awaits further research.

While these studies provide evidence of possible epigenetic carcinogenicity for TCA, it should be pointed out that only one hepatocellular carcinoma was found in the experimental animals used in this study. Further research, using other bioassay systems, is needed to verify the carcinogenetic effects of TCA shown in these studies.

The nearly universal practice of water chlorination assures chronic, widespread human exposure to TCA via drinking water. The implications of low level, chronic human exposure to a possible tumor promoter are unknown. Without more knowledge about environmental levels and toxicity (including carcinogenicity) of TCA and the many other chlorination by-products, no scientifically sound decisions concerning possible biologic hazards related to water chlorination can be made.

In conclusion, this research fulfilled the stated objective of testing the hypothesis that TCA is capable of inducing and/or modulating hepatic carcinognesis in the rat. The parameters used in this research to assess general toxicity indicate that the toxicologic potential for TCA within the rat model is quite low. Furthermore, the results provide valuable carcinogenicity data which should be used in the formulation of any regulatory decisions concerning TCA levels in chlorinated water supplies.

VI. BIBLIOGRAPHY

1. Miller, J.S. and Uden, P.C. Characterization of nonvolatile aqueous chlorination products of humic substances. Environ Sci Technol. 17:150-156 (1983).

2. Coleman, W.E., Munch, J.W., Kaylor, W.H., Streicher, R.P., Ringhand, H.P., and Meler, J.R. Gas chromatography/mass spectroscopy analysis of mutagenic extracts of aqueous chlorinated humic acid. A comparison of the byproducts to drinking water contaminants. Environ Sci Technol. 18:674-681 (1984).

3. Johnson, J.D., Christman, R.F., Norwood, D.L., and Millington, D.S. Reaction products of aquatic humic substances with chlorine. Environ Health Perspect. 46:63-71 (1982).

4. Uden, P.C. and Miller, J.W. Chlorinated acids and chloral in drinking water. J Amer Water Works Assoc. 75:524-527 (1983).

5. Quimby, B.D., Delaney, M.F., Uden, P.C., and Barnes, R.M. Determination of the aqueous chlorination products of humic substances by gas chromatography with microwave emission detection. Anal Chem. 52:259-263 (1980).

6. Rook, J.J. Formation of haloforms during chlorination of natural waters. Water Treat Exam. 23:234-243 (1974).

7. National Cancer Institute Carcinogenesis Program, Division of Cancer Cause and Prevention, Bethesda, MD. Report on carcinogenesis bioassay of chloroform (1976).

8. Cotruvo, J.A. EPA policies to protect the health of consumers of drinking water in the United States. Environ Sci Technol. 15:268-274 (1981).

9. Rook J.J. Chlorination reactions of fulvic acids in natural waters. Environ Sci Technol. 11:478-482 (1977).

10. Oliver, B.G. Chlorinated nonvolatile organics produced by the reaction of chlorine with humic materials. Can Res J. 11:21-22 (1978).

11. Glaze, W.H., Saleh, F.Y., and Kinstley, W. Water Chlorination: Environmental Impact and Health Effects. Vol 3, Ann Arbor Science, Ann Arbor, 1980.

12. Rook, J.J. Water Chlorination: Environmental Impact and Health Effects Vol 3 Ann Arbor Science, Ann Arbor 1980.

13. Windholz, M. (ed). The Merk Index: An Encyclopedia of Chemicals and Drugs. 10th edition, Merck & Co. Inc. Rahway, NJ, 1983.

14. Consulting chemist, personal communication. Sigma Chemical Company. St. Louis, MO 1983.

15. Congressional Information Service. Statistical Reference Index. Washington D.C. 1976 - 1980.

16. Farm Chemical Handbooks. Miester Publishing Company. Willoughby, OH 1977.

17. Martin. Pesticide Manual, Fourth edition.

18. Kearney, P.C. Herbicides: Chemistry, degradation and mode of action. 2nd edition. M. Dekker. Bethesda, MD 1975.

19. ASHP American Hospital Formulary Service. American Society of Hospital Pharmacists. 1983.

20. AMA Drug Evaluations. 4th edition. John Wiley and Sons, New York. 1980.

21. Miller, J.W., Uden, P.C., and Barnes, R.M. Anal Chem. 54:485-488 (1982).

22. Norwood, D.L., Johnson, J.A., Christman, R.F., and Millington, D.S. Water Chlorination: Environmental Impact and Health Effects. Vol 4, Book 1, Ann Arbor Science, Ann Arbor, 1983.

23. Mink, F.L., Coleman, W.E., Munch, J.Y., Kaylor, W.H., and Ringhand, H.P. In vivo formation of halogenated reaction products following peroral sodium hypochlorite. Bull Environ Contam Toxicol. 30:394-399 (1983).

24. Page, G.W. Comparison of groundwater and surface water for levels of contamination by toxic substances. Environ Sci Technol. 15:1475-1481 (1981).

25. Greenberg, M., Anderson, R., Keene, J., Kennedy, A., Page, G.W., and Schowgurow, S. Empirical test of the association between gross contamination of wells with toxic substances and surrounding land use. Environ Sci Technol. 16:14-19 (1982).

26. Roberts, P.V., Reinhard, M., and Volocchi, A.J. Movement of organic contaminants in groundwater: implication for water supply. J Am Water Works Assoc. 74:408-413 (1982).

27. Love, O.T. and Eiero, R.G. Treatment of drinking water containing trichloroethylene and related industrial solvents. J Am Water Works Assoc. 74:413-425 (1982).

28. Lolyd, J.W., Moore, R.M., and Breslin, P. Background information on trichloroethylene. J Occup Med. 17:603-605 (1975).

29. Butler, T.C. Metabolic transformation of trichloroethylene. J Pharmacol Exp Ther. 97:84-92 (1949).

30. Daniel, J.W. The metabolism of 36CL-labelled trichloroethylene and tetrachloroethylene in the rat. Biochem Pharmacol. 12:795-902 (1963).

31. Prout, M.S., Provan, W.M., and Green, T. Species differences in response to trichloroethylene: I. Pharmacokinetics in rats and mice. Toxicol Appl Pharmacol. 79:389-400 (1985).

32. Green, T. and Prout, M.S. Species differences in response to trichloroethylene: II. Biotransformation in rats and mice. Toxicol Appl Pharmacol. 79:401-411 (1985).

33. Millburn, P., Smith, R.L., and Williams, R.T. Biliary excretion of foreign compounds. Biochem J. 105:1275-1281 (1967).

34. Nomiyama, H. and Nomiyama K. Pathway and rate of metabolism of trichloroethylene in rats and rabbits. Ind Health. 17:29-37 (1979).

35. Muller, W.F., Coulston, F., and Korte, F. Comparative metabolism of [14C]trichloroethylene in chimpanzees, baboons and rhesus monkeys. Chemosphere. 11:215-218 (1982).

36. Ziglio, G., Fara, G., Beltramelli, G., and Pregliasco, F. Human environmental exposure to trichloro- and tetrachlorcethylene from water and air in Milan, Italy. Arch Environ Contam Toxicol. 12:57-64 (1983).

37. Sax, N.I. Dangerous Properties of Industrial Materials. 4th edition. Reinhold Publishing Corporation. New York 1980.

38. Driesbach, R.H. Handbook of Poisoning: Prevention, Diagnosis and Treatment. 10th edition. Lange Medical Publications. Las Altos, CA 1980.

39. Gosselin, R.E. Clinical Toxicology of Commercial Products. 5th edition. Williams & Wilkins Company. Baltimore, MD 1984.

40. NIOSH Registry of Toxic EFfects of Chemical Substances. U.S. Department of Health Education and Welfare. Cincinnati, OH 1977.

41. NIOSH Registry of Toxic Effects of Chemical Substances. U.S. Department of Health Education and Welfare. Cincinnati, OH 1976.

42. Correa, M., Calabrese, E.J., and Coler, R.A. Effects of trichloroacetic acid, a new contaminant found from chlorinating water with organic material, on dragonfly nymphs. Bull Environ Contam Toxicol. 34:271-274 (1985).

43. Rapson, W.H., Nazar, M.A., and Butsky, V.K. Mutagenicity produced by aqueous chlorination of organic compounds. Bull Environ Contam Toxicol.24:590-596 (1980).

44. Waskel, L.A. Study of the mutagenicity of anesthetics and their metabolites. Mutation Res. 57:141-153 (1978).

45. Grabb, D.W., Yount, E.A., and Harris, R.A. The metabolic effects of dichloroacetate. Metabolism. 30:1024-1039 (1981).

46. National Cancer Institute. Carcinogenesis Bioassay of Trichloroethylene. CAS no. 79-0106, DHEW Publ No. (NIH) 76-802, Washington, D.C., 1976.

47. National Toxicology Program. National Toxicology Program Draft Report; Abstracts on Nine Chemical Carcinogenesis Animal Bioassays. Chem Regul Rep.6:767-768 (1983).

48. Elcombe, C.R., Pratt, I.S., and Grun, T. The rate of trichloroacetic acid formation determines the species difference in hepatic peroxisome proliferation due to trichloroethylene. Pharmacologist. 24:173 (1982).

49. Elcombe, G.R., Rose, M.S., and Pratt, I.S. Biochemical, histological and ultrastructural changes in rat and mouse liver following the administration of trichloroethylene: Possible relevance to species differences in hepatocarcinogenicity. Toxicol Appl Pharmacol. 79:365-376 (1985).

50. Reddy, J.K., Warren, J., Reddy, M.K., and Lalwani, N.A. Hepatic and renal effect of peroxisome proliferators: Biological implication. Annal NY Acad Sci. 638:81-100 (1982).

51. Lalwani, N.A., Reddy, M.K., Qureshi, S.A., and Reddy, J.K. Development of hepatocellular carinomas and increased peroxisomal fatty acid B-oxidation in rats fed Wy-14,643 in semipurified diet. Carcinogenesis. 2:645-650 (1981).

52. Reddy, J.K., Arzarnoff, D.L., and Hignite, C.E. Hypolipidemic peroxisome proliferators form a novel class of chemical carcinogens. Nature. 283:397-398 (1980).

53. Elcombe, C.R. Species differences in carcinogenicity and peroxisome proliferation trichloroethylene: A biochemical human hazard assessment. Arch Toxicol. (in press).

54. Rhodin, J. Correlation of Ultrastructural Organization and Functions in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney. Doctoral thesis, Karolinska Institutet Stockholm, Sweden 1954.

55. Rouiller, C. and Bernhard, W., "Microbodies" and the problem of mitochondrial regeneration in liver cells. J Biophys Biochem Cytol Suppl. 2:355 (1956).

56. Afzelius, B. The occurrence and structure of microbodies. A comparative study. J Cell Biol. 26:835-840 (1965).

57. Shnitka, T.K. Comparative ultrastructure of hepatic microbodies in some mammals and birds in relation to species difference in uricase activity. J Ultrastruct Res. 16:598-604 (1966).

58. Hruban, Z. and Rechcigl, M. Microbodies and related particles. Morphology, biochemistry and physiology. Int Rev Cytol Suppl. 1:20-32 (1969).

59. deDuve, C., Functions of microbodies (peroxisomes), J Cell Biol. 27: 25A-30 (1965).

60. deDuve, C. and Baudhuin, P. Peroxisomes (microbodies and related particles). Physiol Rev. 46:325-357 (1966).

61. deDuve, C. Exploring cells with a centrifuge. Science. 189:186-201 (1975).

62. Novikoff, A.B. and Goldfischer, S. Visualization of peroxisomes (microbodies) and mitochondria with diaminobenzidine. J Histochem Cytochem. 17:675-680 (1969).

63. Novikoff, A.B., Novikoff, M., Davis, C., and Quintana, N. Studies on microperoxisomes. V. Are microperoxisomes ubiquitous in mammalian cells? J Histochem Cytochem. 21:737-789 (1973).

64. Hruban, Z., Vigil, E., Slesers, A., and Hopkins, E. Microbodies. Constituent organelles of animal cells. Lab Invest. 27:656-681 (1972).

65. Beevers, H. Microbodies in higher plants. Ann Rev Plant Physiol. 30:159-164 (1979).

66. Hruban, Z. and Swift, H. Uricase localization in hepatic microbodies, Science. 146:1316-1329 (1964).

67. Yokota, S. and Fahimi, D. Immunocytochemical localization of catalase in rat liver. J Histochem Cytochem. 29:805-809 (1981).

68. Reddy, J.K., Bendayan, M., and Reddy, M.K. Immunocytochemical localization of catalase, heat-labile enoyl-CoA hydratase and polypeptide PPA-80 in rat liver. Ann NY Acad Sci. 386:495-531 (1982).

69. Reddy, J. and Svoboda, D. Microbodies (peroxisomes) identification in interstitial cells of rodent testis. Lab Invest. 26:184-190 (1972).

70. Beard, M.E. and Novikoff, A.B. Distribution of peroxisomes (microbodies) in the nephron of the rat. A cytochemical study. J Cell Biol. 42:501-507 (1969).

71. deDuve, C. Evolution of the peroxisomes. Ann NY Acad Sci. 168:369-381 (1969).

72. Tolvert, N.E. Metabolic pathways in peroxisomes and glyoxysomes. Ann Rev Biochem. 50:133-157 (1981).

73. Lazarow, P. and deDuve, C. A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug. Proc Natl Acad Sci USA. 73:2043-2046 (1976).

74. Osumi, T. and Hashimoto, T. Enhancement of fatty acyl-CoA oxidizing activity in rat liver peroxisomes by di(2-ethylhexyl)phthalate. J Biochem. 83:1361-1365 (1978).

75. Miyazawa, S., Furuta, S. Osumi, T., Hashimoto, T., and Ui, N. Properties of peroxisomal 3-ketoacyl-CoA thiolase from rat liver. J Biochem. 90:511-517 (1981).

76. Hashimoto, T. Individual peroxisomal B-oxidation enzymes. Ann NY Acad Sci. 386:5-13 (1982).

77. Lazarow, P.B. Functions and Biogenesis of Peroxisomes. in Int Cell Biology. Springer-Verlag, Berlin 1981.

78. Lazarow, P.B. Rat liver peroxisomes catalyze the B-oxidation of fatty acids. J Biol Chem. 253:1522-1528 (1978).

79. Osmundsen, H. Peroxisomal B-oxidation of long chain fatty acids: effect of high fat diets. Ann NY Acad Sci. 386:13-30 (1982).

80. Lazarow, P.B. Three hypolipidemic drugs increase hepatic palmitoyl-CoA oxidation in the rat. Science. 197:580-581 (1977).

81. Lalwani, N.D., Reddy, M.K., Qureshi, S.A., Sirtori, C., Abiko, Y., and Reddy, J.K. Evaluation of selected hypolipidemic agents for the induction of peroxisomal enzymes and peroxisome proliferation in the rat liver. Human Toxicol. 2:27-48 (1983).

82. Reddy, J.K., Warren, J.R., Reddy, M.K., and Lalwani, N.D. Hepatic and renal effects of peroxisome proliferators: Biological impiications. Ann NY Acad Sci. 386: 81-110 (1982).

83. Hess, R., Staubli, W., and Riess, W. Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat. Nature. 208:856-858 (1965).

84. Schulte-Hermann, R. Induction of liver growth by xenobiotic compounds and other stimuli. CRC Crit Rev Toxicol. 33:97-158 (1974).

85. Moody, D.E. and Reddy, J.K. Morphometric analysis of the ultrastructural changes in rat liver induced by the peroxisome proliferator SaH 42-348. J Cell Biol. 71:768-780 (1976).

86. Lazarow, P.B. Assay of peroxisomal B-oxidation of fatty acids. Methods Enxymol. 72:315-321 (1981).

87. Fitzgerald, J.E., Sanyer, J.L., Schardein, J.D., Lahe, R.S., McGurie, E.J., and de la Iglesia, F.A. Carcinogen bioassay and mutagenicity studies with the hypolipidemic agent gemfibrozil. J Natl Cancer Inst. 67:1105-1120 (1981).

88. De La Iglesia, F.A., Pinns, S.M., Luca, J., and McGuire, E.J. Quantitative sterology of peroxisomes in hepatocytes from hyperlipoproteinemic patients receiving gemfibrozil. Micron. 12:97-98 (1981).

89. Reddy, J.K., Qureshi, S.A., Lalwani, N.D., Reddy, M.K., and Moehle, C.M. Induction of hepatic proliferaton in nonrodent species, including primates. Am J Pathol. 114:171-183 (1984).

90. Reddy, J.K., Azarnoff, D.L., and Hignite, C.E. Hypolipidemic peroxisome proliferators form a novel class of chemical carcinogens. Nature. 283:397-398 (1980).

91. Blane, G.F. and Pinaroli, F. Fenofibrate: animal toxicology in relatin to side effects in man. Nour Presse Med. 9:3737-3750 (1980).

92. National Cancer Institute. Bioassay of di(2-ethylhexyl)adipate for possible carcinogenicity. Carcinogenesis Testing Program, U.S. Department of Health and Human Sciences. Publ. No. (NIH)81-1773.

93. National Cancer Institute. Bioassay of di(2-ethylhexyl)phthalate. Carcinogenesis Testing Program, U.S. Department of Health and Human Sciences. Publ. No. (NIH)81-1773.

94. Warren, J.R., Lalwani, N.D., and Reddy, J.K. Properties of hypolipidemic peroxisome proliferators in the lymphocyte [3H]thymidine and Salmonella mutagenesis assays. Cancer Res. 40:36-51 (1980).

95. Warren, J.R., Lalwani, N.D., and Reddy, J.K. Phthalate esters as peroxisome proliferator carcinogens. Environ Health Perspect. 45:35-41 (1982).

96. Reddy, J.K. and Rao, M.S. Enhancement of Wy-24,643, a hepatic peroxisome proliferator of diethylnitrosamine-initiated tumorignesis in the rat. Br J Cancer. 38:537-543 (1978).

97. Mochizuki, Y., Furukawa, K., and Sawada, N. Effects of various concentrations of ethyl-p-chlorophenoxy-isobutyrate (clofibrate) on diethylnitrosamine induced hepatic tumorigenesis in the rat. Carcinogenesis. 3:1027-1034 (1982).

98. Schulte-Hermann, R., Ohde, G., Schippler, J., and Timmermann-Trosiner, K. Enhanced proliferation of putative preneoplastic cells in rat liver following treatment with the tumor promoters phenobarbital, hexachlorocyclohexane, steroid compounds and nafenopin. Cancer Res. 41:2556-2570 (1981).

99. Lalwani, N.D., Reddy, M.K., Qureshi, S.A., and Reddy, J.K. Development of hepatocellular carcinomas and increased peroxisomal fatty acid B-oxidation in rats fed Wy-24,643 in the semi-purified diet. Carcinogenesis. 3:645-650 (9181).

100. Reddy, J.K. and Lalwani, N.D. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. CRC Crit Rev Toxicol. 12:1-58 (1983).

101. Farber, E. The sequential analysis of liver cancer induction. Biochim Biophys Acta. 605:149-190 (1980).

102. Pitot, H.C. and Sirica, A.E. The stages of initiation and promotion in hepatocarcinogenesis. Biochim Biophys Acta. 605:191-216 (1980).

103. Diamond, L., O'Brian, T.G., and Baird, W.M. Tumor promoters and the mechanism of tumor promotion. Adv Cancer Res. 1 (1980).

104. Foerster, E.C., Fahrenkemper, T., Rabe, U., Graf, P., and Sies, H. Peroxisomal fatty acid oxidation as detected by H2O2 production in intact perfused rat liver. Biochem J. 196:705-912 (1981).

105. Inestrosa, N.C., Bronfman, M., and Leighton, L. Detection of peroxisomal fatty acyl-coenzyme A oxidase activity. Biochem J. 182:779-785 (1979).

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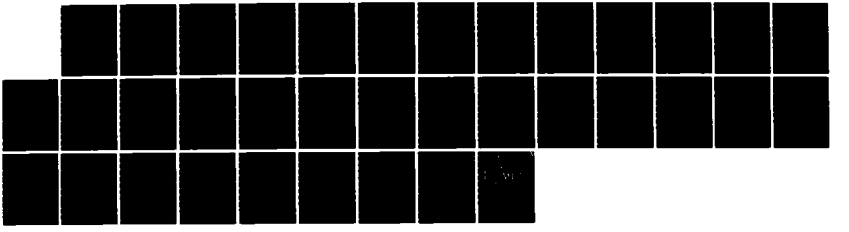
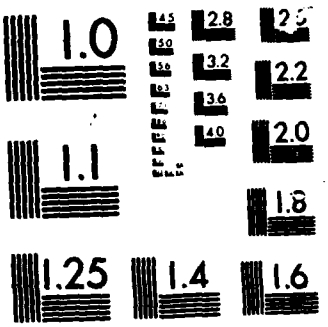




MICROCOPY RESOLUTION TEST CHART

106. Chance, B., Sies, H., and Boveris, A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 59:527-534 (1979).

107. Merletti, F., Heseltine, E., Saracci, R., Simonato, L., Vainio, H., and Wilbourn, J. Target organs for carcinogenicity of chemicals and industrial exposures in humans: a review of resuits in the IARC monographs on the evaluation of the carcinogenic risk of chemicals to numans. Cancer Res. 44:2244-2250 (1984).

108. Farber, E. Chemical carcinogenesis: a current biological perspective. Carcinogenesis. 5:1-5 (1984).

109. Pitot, H.C., Goldsworthy, T., Moran, S., Sirica, A.E., and Weeks, J. Properties of incomplete carcinogens and promoters in hepatocarcinogenesis. Carcinogenesis. 7:85-97 (1982).

110. Montesano, R. and Slaga, T.J. Initiation and promotion in carcinogenesis: an appraisal. Cancer Surveys. 2:613-621 (1983).

111. Farber, E. The multistep nature of cancer development. Cancer Res. 44:4217-4223 (1984).

112. Farber, E. Chemical carcinogenesis: a biologic perspective. Amer. J. Pathol. 106:269-296 (1982).

113. Miller, E.C. Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. Cancer Res. 38:1479-1496 (1978).

114. Singer, B. N-nitrosoalkylating agents: formation and persistence of alkyl derivatives in mammalian nucleic acid as contributing factors in carcinogenesis. J Natl Cancer Inst. 62:1329-1339 (1979).

115. Varshavsky, A. Phorbol ester dramatically increases incidence of methotrexate-resistant mouse cells: possible mechanisms and relevance to tumor promotion. Cell 25:561-572 (1981).

116. Lavi, S. Carcinogen-mediated amplification of specific DNA sequences. J Cell Biochem. 18:149-156 (1982).

117. Boehm, T.L.J. and Drahovsky, D. Alteration of enzymatic methylation of DNA cytosines by chemical carcinogens: a mechanism involved in the initiation of carcinogenesis. J Natl Cancer Inst. 71:429-433 (1983).

118. Wilson, V.L. and Jones, P.A. Inhibition of DNA methylation by chemical carcinogens in vitro. Cell 32:239-246 (1983).

119. Doerfler, W. DNA methylation and gene activity. Annual Rev Biochem. 52:93-124 (1983).

120. Riggs, A.D. and Jones, P.A. 5-Methylcytosine, gene regulation and cancer. Adv Cancer Res. 40:1-30 (1983).

121. Carr, B.I., Reilly, J.G., and Riggs, A.D. 5-Azacytidine: promotion activity for rat hepatocellular carcinoma. IARC Pub. 56. (1984).

122. Columbano, A., Rajalakshmi, S, and Sarma, D.S. Requirement of cell proliferation for the initiation of liver carcinogenesis as assayed by three different procedures Cancer Res. 41:2079-2083 (1982).

123. Cayama E., Tshuda H., Sarma, D.S.R., and Farber, E. Initiation of chemical carcinogenesis requires cell proliferation. Nature. 275:60-62 (1978).

124. Cohen, S., Murasaki, G., Fukushima, S., and Greenfield, R.E. Effect of regenerative hyperplasia on the urinary bladder: carcinogenicity of sodium saccharin and N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. Cancer Res. 42:65-71 (1982).

125. Scarpelli, D.G. and Rao, M.S. Early changes in regenerating hamster pancreas following a single dose of N-nitrosobis(2-hydroxy-propyl)amine (NBOP) administered at the peak of DNA synthesis. Cancer 47:1552-1561 (1981).

126. Bannasch, P., Moore, M.A., Kilmek, F., and Zerban, H. Biological markers of preneoplastic foci and neoplastic nodules in rodent liver. Toxicol Pathol. 10:19-34 (1982).

127. Williams, G.M. Phenotypic properties of preneoplastic rat liver lesions and applications to detection of carcinogens and tumor promoters. Toxicol Pathol. 10:3-10 (1982).

128. Slaga, T.J. Overview of tumor promotion in Animals. Environ Health Perspect. 50:3-14 (1983).

129. Schulte-Hermann, R., Ohde, G., and Schuppler, J. Enhanced proliferation of putative preneoplastic cells in rat liver following treatment with tumor promoters phenobarbital, hexachlorocylohexane, steroid compounds, and nafenopin. Cancer Res. 41:2556-2562 (1981).

130. Pitot, H.C. and Sirica A.E. The stages of initiation and promotion in hepatocarcinogenesis. Biochim Biophys Acta. 605:191-215 (1980).

131. Boutwell, R.K. Biochemical mechanisms of tumor promotion. In: Carcinogenesis: Mechanisms of Tumor Promotion and Cocarcinogenesis, Vol. 2, (T.J. Slaga, A. Sivak, and R.K. Boutwell, Eds.) Raven Press, New York, 1978, pp. 49-58.

132. Olson, J.E. and Russell, D.H. Prolonged ornithine decarboxylase induction in regnererating carcinogen-treated liver. Cancer Res. 40:4373-4380 (1980).

133. Algranati, I.D. and Goldemberg S.H. Polyamines and their role in protein synthesis. Trends Biochem Sci. 2:272-280 (1977).

134. Avraham, A.K. and Pibl A. Role of polyamines in macromolecular synthesis. Trends Biochem Sci. 6:106-111 (1981).

135. Farwell, D.C., Miguwz, J.B., and Herbst, E.J. Ornithine decarboxylase and polyamines in liver and kidneys of rats on cyclical regimen of protein-free and protein-containing diets. Biochem J. 168:49-56 (1977).

136. Russell, D.H., Byus, C.V., and Manen, C.A. Proposed model of major sequential biochemical events of a trophic response. Life Sci. 19:1297-1306 (1976).

137. Pereira, M.A., Savage, R.E., Herren, S., and Guion, C. Comparison of enhancement of GGTase-postive foci and induction of ornithine decarboxylase in rat liver by barbiturates. Carcinogenesis 3:147-150 (1982).

138. Enomoto K., Ying, T.S., Griffin, M., Farber, E. Immunohistochemical study of epoxide hydrolase during experimental liver carcinogenesis. Cancer Res. 41:3281-3287 (1981).

139. Farber, E. and Enomoto, K. Kinetics of phenotypic maturation or remodeling of hyperplastic nodules during liver carcinogenesis. Proc Am Assoc Cancer Res. 22:124-126 (1981).

140. Farber, E. The biology of carcinogen-induced hepatocyte nodules and related liver lesions in the rat. Toxicol, Pathol. 10:197-205 (1982).

141. Comings, D.E. A general theory of carcinogenesis. Proc Natl Acad Sci USA. 70:3324-3328 (1973).

142. Brugge, J.S. and Erikson, R.L. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) 269:346-348 (1977).

143. Stehelin, D., Varmus, H.E., Bishop, J.M., and Vogt, P. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature (London) 260:170-173 (1976).

144. Collett, M.S., Erikson, E., Purchio, A.F., Brugge, J., and Erikson, R.L. A normal cell protein similar in structure and function the avian sarcoma virus gene transforming product. Proc Natl Acad Sci USA. 76:3159-3163 (1979).

145. Sefton, B.M., Hunter, T., and Beemon, K. Relationship of polypeptide products of the transforming gene of Rous sarcoma virus and the homolog gene of vertebrates. Proc Natl Acad Sci USA> 77:2059-2063 (1980).

146. Coffin, J.M., Varmus, H.E., Bishop, J.M., Essex, M., Hardy, W.D., Martin, G.S., Rosenberg, N.E., Scolnick, E.M., Weinberg, R.A., and Vogt, P.K. Proposal for naming host cell-derived inserts in retrovirus genomes. J Virol. 40:953-957 (1981).

147. Karess, R.E., Hayward, W.S., and Hanafusa, H. Cellular information in the genome of recovered avian sarcoma virus directs the synthesis of transforming protein. Proc Natl Acad Sci USA. 76:3154-3158 (1979).

148. Karess, R.E. and Hanafusa, H. Viral and cellular src genes contribute to the structure of recovered avian sarcoma virus transforming protein. Cell 24:155-164 (1981).

149. Shih, C., Shilo, B., Goldfarb, M.P., Dannenberg, A., and Weinberg, R.A. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. Proc Natl Acad Sci USA. 76:5714-5718 (1979).

150. Sukumar, S., Notario, V., Martin-Zanca, D., and Barbacid, M. Induction of mammary carcinomas in rats by nitroso-methyl-urea involves the malignant activation of the H-ras 1 locus by single point mutations. Nature 306:658-661 (1983).

151. Balmain, A., Ramsden, M., Bowden, G.T., and Smith, J. Activation of the mouse cellular H-ras gene in chemically induced benign skin papillomas. Nature 307:658-660 (1984).

152. Sukumar, S., Pulciani, S., Doniger, J., DiPaolo, JaA., Evans, C.H., Zbar, B., and Barbacid, M. Transforming ras gene in tumorigenic guinea pig cell lines initiated by diverse chemical carcinogens. Science (1984).

153. Murray, M., Schilo, B., Shih, C., Cowing, D., Hsu, H., and Weinberg, R.A. Three different human tumor cell lines contain different oncogenes. Cell 25:355-361 (1981).

154. Shih, C., Padhy, L.C., Murray, M., and Weinberg, R.A. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. Nature (London) 290:261-264 (1981).

155. Shilo, B. and Weinberg, R.A. Unique transforming gene in carcinogen-transformed mouse cells. Nature (London) 289:607-609 (1981).

156. Krontiris, T. and Cooper, G.M. Transforming activity of human tumor DNAs. Proc Natl Acad Sci USA. 78:1181-1184 (1981).

157. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 1-31. Lyon, International Agency for Research on Cancer, 1972-1983

158. Merletti, F., Heseltine, E., Saracci, R., Simonato, L., Vainio, H., and Wilbourn, J. Target organs for carcinogenicity of chemicals and industrial exposures in humans: A review of results in the IARC monographs on the evaluation of the carciogneic risk of chemicals to humans. Cancer Res. 44:2244-2250 (1984).

159. Miller, J.A. and Miller, E.C. The metabolic activation of carcinogenic aromatic amines and amides. Prog Exp Tumor Res. 11:273-301 (1969).

160. Miller, E.C. Some current perspectives on chemical carinogenesis in humans and experimental animals: Presidential address. Cancer Res. 38:1479-1496 (1978).

161. Haran, N. and Berenblum, I. The induction of the initiating phase of skin carcinogenesis in the mouse by oral administration of urethane. British J Cancer 10:57-60 (1956).

162. Rossi, L., Revera, M., Repetti, G., and Santi, L. Long-term administraton of DDT or phenobarbital-Na in Wistar rats. Int J Cancer 19:179-185 (1977).

163. Williams, G.M. Classification of genotoxic and epigenetic hepatocarcinogens using liver culture assays. Annals NY Acad Sci. 349:273-282 (1980).

164. Marshall, E. EPA's high risk carcinogen policy. Science 218:975-978 (1982).

165. Williams, G.M. Epigenetic effect of liver tumor promoters and implications for health effect. Environ Health Perspect. 50:177-183 (1983).

166. Sasaki, T. and Yoshida, T. Experimentelle erzeugung des lebercarcinoms durch futterung mit O-amindoazotouol. Virchows Arch. 295:175-200 (1935).

167. Pitot, H.C. and Sirica, A.E. The stages of initiation and promotion in hepatocarcinogesis. Biochim Biophys Acta. 605:191-215 (1980).

168. Farber, E. and Ichinose, H. Acta Unio Inter Contra Cancrum. 15:152-153 (1959).

169. Goldfarb, S. and Zak, R.G. Role of injury and hyperplasia in the induction of hepaocellular carcinoma. JAMA. 178:729-731 (1961).

170. Peraino, C., Fry, R.J., Staffeldt, E., and Kisieleski, W.E. Effects of varing the exposure to phenobarbital on its enhancement of 2-acetylamino-fluorene induced hepatic tumorigenesis in the rat. Cancer Res. 33:701-705 (1973).

171. Gossner W. and Friedrich-Fresksa, H. Histochemische untersuchungen uber die glukose-6-phosphatase in der rattenleber wahrend der cancerisierung durch nitrosamine. Z Naturforsch 19:862-863 (1964).

172. Schauer, A. Histochemische und biochemische untersuchunger bei der cancerisierung mit diaethylnitrosamin an der rattenleber. Verh Dtsch Ges Pathol. 50:344-347 (1966).

173. Schauer, A. and Kunze, E. Enzymhistochemische und autoradiographische untersuchunger wahrend der cancerisierung der rattenleber mit diathylnitrosamin. Z Krebsforsch 70:252-266 (1968).

174. Taper, H.S., Fort, L., and Brucher, J.M. Histochemical activity of alkaline and acid nucleases in the rat liver parenchyma during N-nitrosomorpholine carcinogenesis. Cancer Res. 31:913-916 (1971).

175. Kitagawa, T. and Pitot, C. The regulation of serine dehydratase and glucose-6-phosphatase in hyperplastic nodules of rat liver during diethylnitrosamine and N-2-fluorenylacetamide feeding. Cancer Res. 35:1075-1084 (1975).

176. Epstein S., Ito, N., Merkow, L., and Farber, E. Cellular analysis of liver carcinogenesis: the induction of large hyperplastic nodules in the liver with 2-fluorenylacetamide or ethionine and some aspects of their morphology and glycogen metabolism. Cancer Res. 27:1702-1711 (1967).

177. Sell, S. Distribution of alpha-fetoprotein and albumin-containing cells in the livers of Fischer rats fed four cycles of N-2-fluorenylacetamide. Cancer Res. 36:3107-3113 (1978).

178. Pitot, H., Barsness, L., Goldsworthy, T., and Kitagawa T. Biochemical characterization of stages of hepatocarciogenesis after a single dose of diethylnitrosamine. Nature 271:456-458 (1978).

179. Williams G.M. and Yamamoto, R.S. Absence of stainable iron from preneoplastic and neoplastic lesions in rat liver with 8-hydroxy-quinoline-induced siderosis. J Natl Cancer Inst. 49:685-692 (1972).

180. Farber, E. The sequential analysis of liver cancer induction. Biochim Biophys Acta. 605:149-166 (1980).

181. Farber, E. The biochemistry of preneoplastic liver: a common metabolic pattern in hepatocyte nodules. Can J Biochem Cell Biol. 62:486-494 (1984).

182. Fila, S., Mohindru, A., Kettering, W.G., Fiala, A.E., and Morris, H.P. Glutathione and gamma glutamyl transpeptidase in rat liver during chemical carcinogenesis J Natl Cancer Inst. 57:591-598 (1976).

183. Cameron, R., Kellen, J., Kolin, A., Malkin, A., and Farber, E. Gamma-glutamyltransferase in putative premalignant liver cell populations during hepatocarcinogenesis. Cancer Res. 38:823-829 (1978).

184. Laishes, B.A., Ogawa, K., Roberts, E., and Farber, E. Gamma-glutamyl transpeptidase: a positive marker for cultured rat liver cells derived from putative premalignant and malignant lesions. J Natl Cancer Inst. 60:1009-1016 (1978).

185. Roomi, M.W., Sharma, R., Ho, R., Eriksson, L., Farber, E., and Murray, R.K. The polypeptides of the cytosol of hepatocyte nodules may exhibit a unique electrophoretic pattern. Proc Am Assoc Cancer Res. 24:90 (1983).

186. Eriksson, L.C., Sharma, R.N., Roomi, M.W., Ho, R.K., Farber, E., and Murray, R.K. A characteristic eletrophoretic pattern of cytosolic polypeptides from hepatocyte nodules generated during liver carcinogenesis in several models. Biochem Biophys Res Commun. 117:740-745 (1983).

187. Tsuda, H., Lee, G., and Farber, E. Induction of resistant hepatocytes as a new principle for a possible short-term in vivo test for carcinogens. Cancer Res. 40:1157-1164 (1980).

188. Farber, E. The biology of carcinogen-induced hepatocyte nodules and related liver lesions in the rats. Toxicol Pathol. 10:197-201 (1982).

189. Farber, E. and Cameron, R. The sequential analysis of cancer development. Adv Cancer Res. 35:125-226 (1980).

190. Solt, D.B. and Farber, E. New principle for the analysis of chemical carcinogenesis. Nature (London). 263: 702-703 (1976).

191. Appel, K.E., Richart, R., and Kunz, W. Influences of inducers and inhibitors of the microsomal monooxygenase system on the alkylating intensity of dimethylnitrosamine in mice. J Cancer Res Clin Oncol. 94:47-61 (1979).

192. Peraino, C., Fry, R.J.M., and Staffeldt, E. Reduction and enhancement by phenobarbital of hepatocarcinogenesis induced in rat by 2-acetylaminofluorene. Cancer Res. 31:1506-1512 (1971).

193. Goldsworthy, T., Campbell, H.A., and Pitot, H.C. The natural history and dose-response characteristics of enzyme-altered foci in rat liver following phenobarbital and diethylnitrosamine administration. Carcinogenesis 5:67-71 (1984).

194. Scherer, E. and Emmelot, P. Kinetics of induction and growth of precancerous liver-cell foci and liver tumor formation by diethylnitrosamine in the rat. Eur J Cancer 11:689-701 (1975).

195. Scherer, E. and Emmelot, P. Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. Cancer Res. 36:2544-2554 (1976).

196. Popp, J.A. and Leonard, T.B. The use of in vivo hepatic initiation-promotion systems in understanding the hepatocarcinogenesis of technical grade dinitrotoluene. Toxicol Pathol. 10:190-194 (1982).

197. Pereira, M.A., Herren, S.L., Britt, A.L., and Khoury, M.M. Initiation/promotion bioassay in rat liver: use of gamma glutamyltranspeptidase-postive foci to indicate carcinogenic activity. Toxicol Pathol. 10:11-18 (1982).

198. Tatematsu, M., Hasegawa, R., Imaida, K., Tsuda, H., and Ito, N. Survey of various chemicals for initiating and promoting activities in a short-term in vivo system based on generation of hyperplastic liver nodules in rats. Carcinogenesis 4:381-386 (1983).

199. Schulte-Hermann, R., Timmerman-Trosiener, I., and Schuppler, J. Response of liver foci in rats to hepatic tumor promoters. Toxicol Pathol. 10:63-68 (1982).

200. Herren, S.L. and Pereira, M.A. Tumor promotion in rat liver. Environ Health Perspect. 50:123-129 (1983).

201. Goldfarb, S. and Pugh, T.D. Enzyme histochemical phenotypes in primary hepatocellular carcinomas. Cancer Res. 41:2092-2095 (1981).

202. Pugh, T.D. and Goldfarb, S. Quantitative histochemical and autoradiographic studies of hepatocarcinogenesis in rats fed 2-acetylaminofluorene followed by phenobarbital. Cancer Res. 38:4450-4457 (1978).

203. Ogawa, K., Solt, D.B., and Farber, E. Phenotypic diversity as an early property of putative preneoplastic hepatocyte populations in liver carcinognesis. Cancer Res. 40:725-733 (1980).

204. Hirota, N. and Williams, G.M. The sensitivity and heterogeneity of histochemical markers for altered foci involved in liver carcinogenesis. Am J Pathol. 95:317-324 (1979).

205. Tatematsu, M., Takano, T., Hasegawa, R., Imaida, K., Nakanowatari, J., and Ito, N. A sequential quantitative study of the reversibility or irreversibility of liver hyperplastic nodules in rats exposed to hepatocarcinogens. Gann. 71:843-855 (1980).

206. Williams, G.M. and Watanabe, K. Quantitative kinetics of development of N-2-fluorenylacetamide induced altered (hyperplastic) hepatocellular foci resistant to iron accumulation and of their reversion or persistance following removal of carcinogen. J Natl Cancer Inst. 61:113-121 (1978).

207. Williams, G.M. and Watanabe, K. Enhancement of rat hepatocellular-altered foci by the liver tumor promoter phenobarbital: evidence that foci are precursors of neoplasms and that the promoter acts on carcinogen-induced lesion. J Natl Cancer Inst. 61:1311-1314 (1978).

208. Kitagawa, T. Sequential phenotypic changes in hyperplastic areas during hepatocarcinogenesis in the rat. Cancer Res. 36:2534-2539 (1976).

209. Moore, M., Hacker, H., and Bannasch, P. Phenotypic instability in focal and nodular lesions induced in a short term system in the rat liver. Carcinogenesis 4:595-603 (1983).

210. Jensen, R.K., Sleight, S.A., Goodman, J.I., Aust, S.D., and Trosko, J.E. Polybrominated biphenyls as promoters in experimental hepatocarcinogenesis in rats. Carcinogenesis 3:1183-1186 (1982).

211. De La Iglesia, F.A. and Farber, E. Hypolipidemics carcinogenicity and extrapolation of experimental results for human safety assessments. Toxicol Pathol. 10:152-170 (1982).

212. Higgins, G.M. and Anderson, R.M. Experimental pathology of the liver. Arch Pathol. 12:186-202 (1931).

213. Rutenburg, A.M., Hwakyu, K., Fischbein, J.W., Hanker, J.S., Wasserkrug, H.L., and Seligman, A.M. Histochemical and ultrastructural demonstration of gamma-glutamyl transpeptidase activity. J Histochem Cytochem. 17:517-527 (1969).

214. Omura, T. and Sato, R. The carbon monoxide-binding pigment of liver microsomes. J Biol Chem 239:2370-2378 (1964).

215. Prough, R.A., Burke, M.D., and Mayer, R.T. Direct fluorometric methods for measuring mixed-function oxidase activity. Methods Enzymol. 52:372-377 (1978).

216. Werringloer, J. Assay of formaldehyde generated during microsomal oxidation reactions. Methods Enzymol. 52: 297-302 (1978).

217. Bethell, D.R. and Pegg, A.E. Effects of diamines on ornithine decarboxylase activity in control and virally transformed mouse fibroblasts. Biochem J. 180:87-94 (1979).

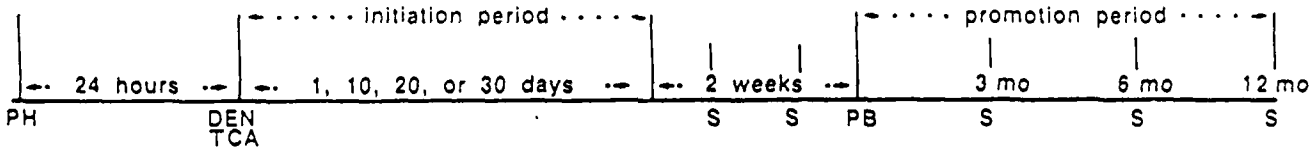
218. Savage, R.E., Westrich, C., Guion, C., and Pereira, M.A. Chloroform induction of ornithine decarboxylase activity in rats. Environ Health Perspect. 46:157-162 (1982).

219. Savage, R.E., Herren, S., Pereira, M.A. Effect of alpha-difluoromethylornithine, an irreversible ornithine decarboxylase inhibitor, on formation of GGTase-positive foci. J Toxicol Enivron Health 11:637-641 (1983).

220. Hanigan, M.H. and Pitot H.C. Gamma-glutamyl transpeptidase - its role in hepatocarcinogenesis. Carcinogenesis 6:165-172 (1985).

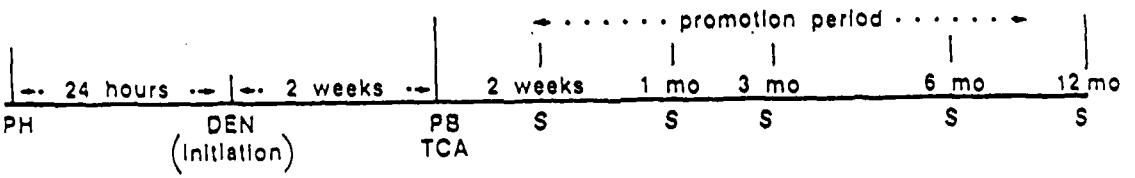
FIGURES

Figure 1. Experimental Design For Initiation Study



PH = partial hepatectomy
DEN = Diethylnitrosamine
PB = Phenobarbital
TCA = Trichloroacetic Acid
S = Sampling interval

Figure 2. Experimental Design for Promotion Study



PH = partial hepatectomy
DEN = Diethylnitrosamine
PB = *Phenobarbital*
TCA = Trichloroacetic Acid
S = Sampling interval

TABLES

Table 1. Experimental Initiation Protocol

Group	A	B	C	D	E	F	G
PH	+	+	+	+	+	-	+
Initiator	DEN[a]	TCA[b]	TCA[c]	TCA[c]	TCA[c]	TCA[c]	--
Days dosed	1	1	10	20	30	30	--
Promotor	PB[d]	PB[d]	PB[d]	PB[d]	PB[d]	PB[d]	PB[d]

PH = partial *hepatectomy*, *DEN* = *diethylnitrosamine*, PB = sodium phenobarbital, TCA = trichloroacetic acid

[a] DEN single oral gavage, 10 mg/kg in distilled H_2O

[b] TCA single oral gavage, 1500 mg/kg in distilled H_2O

[c] TCA in drinking H_2O, 5000 ppm

[d] PB in drinking H_2O, 500 ppm

Table 2. Experimental Promotion Protocol

Group	M	N	O	P	Q	R	S
PH	+	+	+	+	-	+	+
Initiator	DEN[a]	DEN[a]	DEN[a]	DEN[a]	--	--	DEN[a]
Promotor	PB[b]	TCA[c]	TCA[b]	TCA[e]	TCA[c]	--	--

PH = partial hepatectomy, DEN = diethylnitrosamine, PB = sodium phenobarbitol, TCA = trichloroacetic acid

[a] DEN single oral gavage, 10 mg/kg in distilled H_2O

[b] PB in drinking H_2O, 500 ppm

[c] TCA in drinking H_2O, 50 ppm

[d] TCA in drinking H_2O, 500 ppm

[e] TCA in drinking H_2O, 5000 ppm

Table 3. TCA Initiation -- GGT-Positive Foci

Group	Treatment*	N[b]	mean foci/cm^2 (± SEM)			mean foci area (mm^2)		
			3-month	6-month	12-month	3-month	6-month	12-month
A	PH/DEN/PB	6 (12)	2.05±.18[a]	9.93±.71[a]	9.146±.12[a]	.027±.017	.041±.025	.084±.053
B	PH/1 dose/PB	6 (12)	.05±.18	.32±.71	.409±.12	.020±.00	.022±.014	.050±.032
C	PH/10 days/PB	6 (12)	.08±.18	.28±.71	.767±.12	.015±.014	.018±.010	.057±.037
D	PH/20 days/PB	6 (12)	.07±.18	.30±.71	.728±.12	.015±.008	.021±.011	.051±.029
E	PH/30 days/PB	6 (12)	.06±.18	.33±.71	.807±.12	.013±.004	.020±.009	.060±.030
F	/30 days/PB	6 (12)	.10±.18	.49±.71	.752±.12	.012±.003	.019±.009	.059±.052
G	PH/PB	4 (9)	.07±.22	.14±.86	.772±.22	.023±.00	.025±.004	.048±.033
Negative Control		-- (7)	--	--	.420±.47	--	--	.040±.016

* PH = partial hepatectomy, DEN = diethylnitrosamine, PB = sodium phenobarbital, TCA = trichloroacetic acid, SEM = standard error of least squares means

[a] Significantly greater than groups B, C, D, E, F and G by least squares means comparisons ($P \leq 0.05$). All other comparisons were not significant.

[b] Numbers of animals at 12-month sampling in parentheses.

Table 4. TCA Promotion -- GGT-Positive Foci

Group	Treatment*	N[d]	mean foci/cm^2 (± SEM)			mean foci area (mm^2)		
			3-month	6-month	12-month	3-month	6-month	12-month
M	PH/DEN/PB	6 (12)	1.65±.23[a]	7.61±.72[a]	11.20±.36	.031±.015	.049±.034	.093±.057
N	PH/DEN/50 ppm TCA	6 (12)	.71±.16[b]	1.82±.32[c]	4.33±.36	.019±.008	.035±.023	.071±.055
O	PH/DEN/500 ppm TCA	6 (12)	.39±.16	1.63±.32[c]	3.94±.36	.021±.008	.032±.019	.078±.071
P	PH/DEN/5000 ppm TCA	6 (12)	.70±.16[b]	2.45±.32[c]	4.11±.36	.022±.008	.033±.020	.075±.067
Q	5000 ppm TCA	6 (12)	.03±.16	.03±.32	.31±.36	.015±.005	.020±.008	.048±.050
R	PH	4 (6)	.23±.20	.41±.39	.56±.50	.016±.004	.021±.007	.035±.012
S	PH/DEN	4 (5)	.05±.20	.30±.39	.75±.55	.014±.005	.023±.005	.053±.069
Negative Control		-- (7)	--	--	.42±.47	--	--	.040±.016

* PH = partial hepatectomy, DEN = diethylnitrosamine, PB = sodium phenobarbital TCA = trichloroacetic acid, SEM = standard error of least squares means

[a] Significantly greater than groups N, O, P, Q, R and S by least squares means comparisons ($P \leq 0.05$).

[b] Significantly greater than groups Q and S by least squares means comparisons ($P \leq 0.05$). Group M excluded from comparisons.

[c] Significantly greater than groups Q, R and S by least squares means comparison ($P \leq 0.05$). Group M excluded from comparisons.

[d] Numbers of animals at 12-month sampling in parentheses.

Table 5. TCA Promotion -- ^{14}C Palmitoyl CoA Oxidation

Group	Treatment*	N	mean μm/min/g liver (± SEM) 2-week	1-month	3-month	6-month	12-month
M	PH/DEN/PB	6	.37 ± .01[a]	.36 ± .02[a]	.45 ± .04[a]	.54 ± .02[a]	.46 ± .01[a]
N	PH/DEN/50 ppm TCA	6	.49 ± .02	.53 ± .02	.59 ± .03	.67 ± .02	.56 ± .01
O	PH/DEN/500 ppm TCA	6	.55 ± .01	.49 ± .01	.58 ± .01	.64 ± .02	.55 ± .01
P	PH/DEN/5000 ppm TCA	6	.61 ± .01[b]	.64 ± .01[b]	.70 ± .02[b]	.76 ± .02[b]	.62 ± .01[b]
Q	5000 ppm TCA	6	.59 ± .02[b]	.63 ± .01[b]	.66 ± .02[b]	.77 ± .02[b]	.60 ± .01[b]
R	PH	4	.52 ± .01	.53 ± .01	.58 ± .02	.66 ± .02	.52 ± .01
S	PH/DEN	4	.56 ± .02	.51 ± .02	.62 ± .01	.68 ± .02	.53 ± .01
Negative Control[c]		7					.53 ± .01

* PH = partial hepatectomy; DEN = diethylnitrosamine; PB = sodium phenobarbitol; TCA = trichloroacetic acid; SEM = standard error of least squares means

[a] Significantly lower than groups N, O, P, Q, R and S by least squares means comparisons ($P \leq 0.05$)

[b] Significantly greater than groups M, N, O, R and S by least squares means ($P \leq 0.05$)

[c] Only sampled at 12 month interval

Table 6. Clofibrate - ^{14}C Palmitoyl CoA Oxidation and Liver Weights

Treatment	N	Mean μm/min/g liver (± SEM)	Liver weight expressed as a percentage of body weight
Clofibrate[a]	3	3.570 ± 0.193[b]	6.28 ± 0.11[c]
Corn oil	3	0.573 ± 0.193	4.87 ± .011

[a] 250 mg/kg clofibrate in corn oil via oral gavage, once daily for 14 days.

[b] Significantly different at $P \leq 0.0005$

[c] Significantly different at $P \leq 0.001$

Table 7. TCA Initiation -- ^{14}C Palmitoyl CoA Oxidation

Group	Treatment*	N	mean μm/min/g liver (± SEM)				
			first	second	3-month	6-month	12-month
A	PH/DEN/PB	6	.49 ± .03	.50 ± .02	.40 ± .01	.57 ± .02	.53 ± .02
B	PH/1 dose/PB	6	.59 ± .04	.44 ± .03	.42 ± .02	.55 ± .02	.51 ± .02
C	PH/10 days/PB	6	.54 ± .02	.57 ± .08	.42 ± .02	.58 ± .02	.53 ± .02
D	PH/20 days/PB	6	.55 ± .03	.48 ± .01	.43 ± .03	.55 ± .02	.53 ± .02
E	PH/30 days/PB	6	.59 ± .03	.48 ± .01	.37 ± .03	.55 ± .02	.52 ± .02
F	30 days/PB	6	.57 ± .02	.48 ± .01	.37 ± .03	.59 ± .02	.54 ± .02
G	PH/PB	4	.55 ± .01	.58 ± .05	.45 ± .01	.57 ± .02	.53 ± .02
Negative Control[a]		7					.52 ± .02

* PH = partial hepatectomy; DEN = diethylnitrosamine; PB = sodium phenobarbitol; TCA = trichloroacetic acid; SEM = standard error of least squares means

[a] Only sampled at 12 month interval

Table 8. TCA Initiation -- Organ/Body Weights

Group	Treatment*	N	mean organ weight as % body weight (± SEM)					
			3-month			6-month		
			spleen	liver	kidney	spleen	liver	kidney
A	PH/DEN/PB	6	.22±.15	5.20±.22	.43±.02	.19±.01	5.20±16	.43±.02
B	PH/1 dose/PB	6	.22±.15	5.70±.22	.44±.02	.19±.01	5.17±16	.40±.02
C	PH/10 days/PB	6	.22±.15	5.96±.22	.52±.02	.20±.01	5.04±16	.41±.02
D	PH/20 days/PB	6	.26±.15	5.51±.22	.50±.02	.20±.01	4.91±16	.40±.02
E	PH/30 days/PB	6	.21±.15	5.44±.22	.49±.02	.21±.01	5.04±16	.43±.02
F	30 days/PB	6	.20±.15	5.44±.22	.49±.02	.19±.01	4.97±16	.43±.02
G	PH/PB	4	.22±.18	5.83±.27	.44±.03	.22±.01	4.76±20	.45±.02

Group	Treatment*	N	mean organ wt as % body wt (± SEM)		
			12-month		
			spleen	liver	kidney
A	PH/DEN/PB	6	.19±.01	4.01±.16	.38±.01
B	PH/1 dose/PB	6	.21±.01	3.69±.16	.37±.01
C	PH/10 days/PB	6	.19±.01	3.74±.16	.41±.01
D	PH/20 days/PB	6	.18±.01	3.40±.16	.36±.01
E	PH/30 days/PB	6	.19±.01	3.64±.16	.38±.01
F	30 days/PB	6	.18±.01	4.02±.16	.40±.01
G	PH/PB	4	.19±.01	3.76±.18	.38±.01

* PH = partial hepatectomy; DEN = diethylnitrosamine; PB = sodium phenobarbitol; TCA = trichloroacetic acid; SEM = standard error of least squares means

Table 9. TCA Promotion -- Organ/Body Weights

Group	Treatment*	N	mean organ weight as % body weight (± SEM)					
			3-month			6-month		
			spleen	liver	kidney	spleen	liver	kidney
M	PH/DEN/PB	6	.23±.01	5.47±.14[a]	.53±.02	.19±.13	5.06±.15[a]	.42±.02
N	PH/DEN/50 ppm TCA	6	.23±.01	3.92±.14	.52±.02	.18±.13	3.76±.15	.40±.02
O	PH/DEN/500 ppm TCA	6	.20±.01	4.12±.14	.55±.02	.17±.13	4.41±.15	.46±.02
P	PH/DEN/5000 ppm TCA	6	.22±.01	4.25±.14	.54±.02	.19±.13	4.41±.15	.48±.02
Q	5000 ppm	6	.20±.01	4.19±.14	.58±.02	.18±.13	4.44±.15	.53±.02
R	PH	4	.21±.02	3.73±.17	.53±.02	.18±.15	3.83±.19	.46±.02
S	PH/DEN	4	.20±.02	3.90±.17	.55±.02	.15±.15	3.96±.19	.49±.02

Group	Treatment*	N	mean organ wt as % body wt (± SEM)		
			12-month		
			spleen	liver	kidney
M	PH/DEN/PB	6	.22±.01	4.17±.20	.38±.03
N	PH/DEN/50 ppm TCA	6	.23±.01	3.74±.19	.45±.03
O	PH/DEN/500 ppm TCA	6	.22±.01	3.79±.19	.42±.03
P	PH/DEN/5000 ppm TCA	6	.25±.01	4.19±.19	.48±.03
Q	5000 ppm	6	.20±.01	3.92±.19	.50±.03
R	PH	4	.24±.01	3.51±.26	.45±.04
S	PH/DEN	4	.20±.02	4.00±.29	.43±.04
Negative Control		7	.21±.20	3.15±.29	.46±.04

* PH = partial hepatectomy; DEN = diethylnitrosamine; PB = sodium phenobarbitol; TCA = trichloroacetic acid; SEM = standard error of least squares means

a Significantly greater than groups N, O, P, Q, R, and S by least squares means comparisons ($P \leq 0.05$). All other comparisons were not significant

Table 10. TCA - Mammary Tumors at 12 Months

Initiation		Promotion	
Group[a]		Group[a]	
A	3/21[b]	M	4/18[b]
B	4/19	N	3/17
C	1/15	O	2/20
D	2/15	P	2/14
E	3/17	Q	3/20
F	0/13	R	2/8
G	1/9	S	1/6
Neg. Control	1/9	Neg. Control	2/11

[a] See Tables 1 and 2 for explanation of groups and treatments.

[b] Values are expressed as animals with mammary tumors versus total number of animals at risk.

Table 11. TCA - ^{14}C Palmitoyl CoA Oxidation
90-day Subchronic Study

Treatment	N	mean μm/min/g liver (± SEM)
0 ppm	8	0.550 ± 0.014
50 ppm	8	0.518 ± 0.014
500 ppm	8	0.552 ± 0.014
5000 ppm	8	0.635 ± 0.014[a]

[a] $P \leq 0.0005$ level by least squares mean comparisons.

Table 12. TCA - Hepatic P-450 Content
90-day Subchronic Study

Treatment	N	mean nmol P-450/mg microsomal protein (± SEM)
0 ppm	6	0.800 ± 0.029
50 ppm	6	0.707 ± 0.029[a]
500 ppm	6	0.816 ± 0.029
5000 ppm	6	0.862 ± 0.029

[a] $p<0.05$ level by least squares mean comparisons.

Table 13. TCA - Aminopyrine N-Demethylase Activity
90-day Subchronic Study

Treatment	N	mean activity nmol/mg protein/min (± SEM)
0 ppm	2	12.88 ± 1.63
50 ppm	2	14.42 ± 1.63
500 ppm	2	16.70 ± 1.63
5000 ppm	2	17.86 ± 1.63

Means are not significantly different at the $P \leq 0.05$ level.

Table 14. TCA - Hepatic Ethoxy-Coumarin O-de Ethylase Activity 90-day Subchronic Study

Treatment	N	mean activity nmol/mg protein/min (± SEM)
0 ppm	2	0.736 ± 0.113
50 ppm	2	0.796 ± 0.113
500 ppm	2	0.790 ± 0.113
5000 ppm	2	1.050 ± 0.113

Means are not significantly different at the $P \leq 0.05$ level.

Table 15. TCA 90 Day Subchronic Study - Body and Organ Weights
Organ Weight Expressed as Percentage of Body Weight

Trt	N	Beginning Body Wt	N	Ending Body Wt	Kidney Wt	Liver Wt	Thymus Wt	Heart Wt	Spleen Wt
0	10	249.04±4.59	9	435.68±12.98	0.37±0.01	4.07±0.10	0.09±0.01	0.33±0.02	0.22±0.01
50	10	249.13±4.59	8	426.56±13.77	0.37±0.01	4.05±0.10	0.09±0.01	0.31±0.02	0.19±0.01
500	10	242.55±4.59	10	416.38±12.32	0.38±0.01	4.07±0.09	0.10±0.01	0.33±0.02	0.21±0.01
5000	10	250.06±4.59	10	413.96±12.32	0.41±0.01	4.35±0.09	0.08±0.01	0.33±0.02	0.20±0.01

Values are expressed as means ± standard error of the least squares mean.

Table 16. TCA Subchronic (90-Day) Blood Chemistries[a]

Trt[b]	N	BUN (mg/dl)	CREAT (mg/dl)	CA (mg/dl)	PHOS (mg/dl)	GLUC (mg/dl)	CHOL (mg/gl)	TOT PROT (g/dl)	ALBU (g/dl)	CPK (IU/l)	GGT (IU/l)	ALT (IU/l)	ALK PHOS (IU/l)
Initial Values													
0	5	19±1.2	.4±.02	10.7±.3	14.0±.9	137±7.9	70±5	6.1±.3	3.2±.05	1388±110	0	40.9±3.4	288±19.5
50	5	19.1.2	.3±.02	10.3±.3	11.4±.8	123±7.9	66±5	5.6±.3	3.1±.05	1201±70	0	40.4±3.4	320±19.5
500	5	21±1.2	.4±.02	10.8±.3	15.2±.7	127±7.9	74±5	6.0±.3	3.2±.05	1415±70	0	48.9±3.4	316±19.5
5000	5	20±1.2	.5±.02	11.1±.3	15.1±.7	117±7.9	75±5	6.2±.3	3.2±.05	1321±170	0	39.4±3.4	300±19.5
Values at 90 Days													
0	9	29±1.0	.7±.02	12.2±.36	10.2±.45	255±24.5	93±4.9	7.3±.1	3.3±.6	460±312	0	53.9±6.9	212±11.4
50	7	26.1.2	.7±.02	11.7±.41	10.0±.51	211±27.8	71±5.6[c]	7.1±.1	3.3±.7	1450±354	0	50.5±7.9	211±12.9
500	9	24±1.0	.7±.02	11.1±.36	9.6±.45	187±24.5	78±4.9[c]	7.1±.1	3.3±.6	1028±312	0	59.9±6.9	196±11.4
5000	10	27±1.0	.7±.02	11.1±.36	9.2±.42	205±23.3	66±4.6[c]	7.0±.1	3.4±.6	524± 29	0	79.4±6.6[c]	216±10.8

[a] Values are expressed as means ± SEM. BUN = blood urea nitrogen; CREAT = creatine; CA = calcium; PHOS = phosphorus; GLUC = glucose; CHOL = cholesterol; TOT PROT = total protein; ALBU = albumen, CPK = creatine phosphokinase; GGT = gamma-glutamyl transpeptidase; ALT = alanine aminotransferase; ALK PHOS = alkaline phosphatase.

[b] TCA in drinking water at 0, 50, 500, or 5000 ppm.

[c] $P \leq .05$

Table 17. TCA - ^{14}C Ornithine Decarboxylase Activity

Treatment	N	PMole ^{14}C O_2/30 min/mg protein	
		5 hours	18 hours
Control	4	0.82±8.33	2.54±6.96[b]
TCA[a]	4	56.96±8.33	66.20±6.96[c]

[a] Single oral gavage of 1500 mg/kg TCA in distilled H_2O.

[b] Significantly different at $P \leq 0.005$.

[c] Significantly different at $P \leq 0.001$.

Table 18. Daily Water Intake

Treatment	3 weeks[a]		3 months[a]		6 months[a]	
	ml/kg	(mg/kg)	ml/kg	(mg/kg)	ml/kg	(mg/kg)
PB (500 ppm)	92.97	(46.49)	90.85	(45.43)	84.05	(42.03)
TCA (50 ppm)	86.58	(4.33)	81.40	(4.07)	57.78	(2.89)
TCA (500 ppm)	99.31	(49.66)	87.23	(43.62)	59.12	(29.56)
TCA (5000 ppm)	62.50	(312.50)	61.28	(306.40)	55.39	(276.95)
Distilled H_2O	109.38	--	84.11	--	60.98	--

[a] Values represent means of 4 animals measured daily for 5 days.

FROM: CIMI

28 January 1986

SUBJECT: Dissertation Transmittal - Capt Michael J. Parnell, USAF, BSC

TO: NR

1. The attached dissertation entitled "Toxicologic Evaluation of Trichloroacetic Acid: Effects on Rat Liver Peroxisomes and Enzyme-Altered Foci" by Capt Michael J. Parnell, USAF, BSC is forwarded for your review and action. I would recommend the following individual as reviewer:

Col Philip E. Smead, USAF, BSC
HQ USAF/SGB
Bolling AFB DC 20332

2. Thank you for your assistance.

GARY D. McMANN, Capt, USAF, MSC
Program Manager, Health Care Educ Div
Civilian Institution Programs

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Dissertation

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